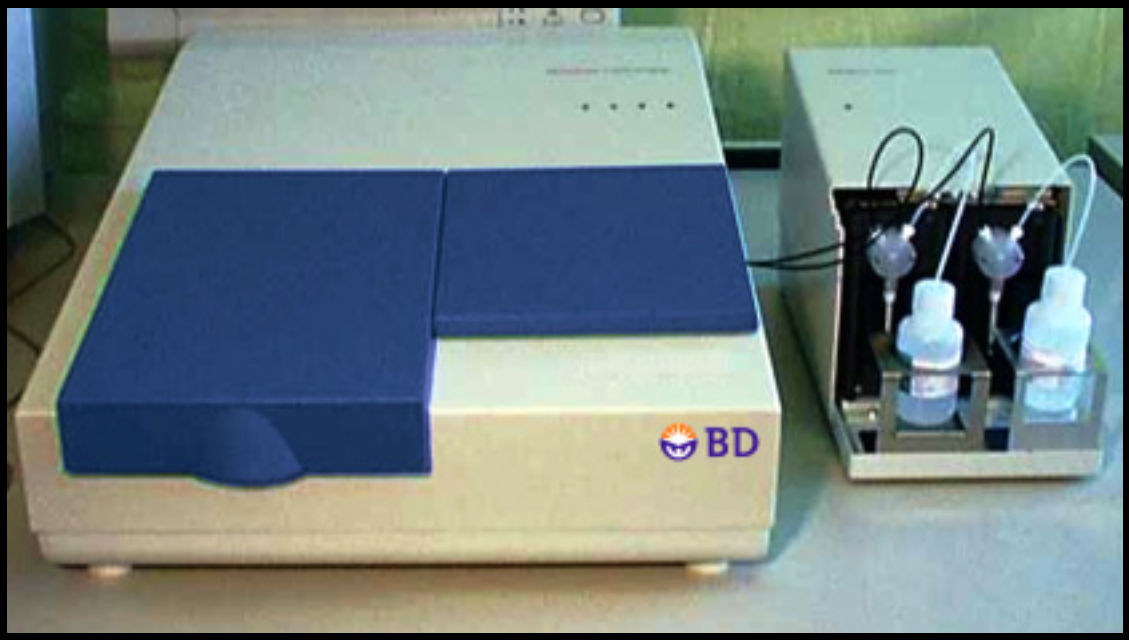

MONOLIGHT 3096

Microplate Luminometer

Operating Manual

BD Biosciences www.bdbiosciences.com

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Revised 6/19/01

Contents

Typographical Conventions

To make it easy for you to work with this manual and to use the software, we will use the following typographical conventions throughout this manual:

Button labels are printed in bold typeface inside angular brackets. Example: <**OK**>, <**Start Quick Measurement**>

Menu and option titles are printed in bold typeface inside square brackets. Example: [**File**], [**Options**]

Actions are symbolized by ❑.

Enumerations are symbolized by ●.

1. Safety Instructions

The **MONOLIGHT 3096** as well as the **injector unit** were manufactured in accordance with the safety requirements for electronic and medical measuring systems. If the law states regulations on the installation and/or operation of sample measuring systems, then it is the operator's responsibility to adhere to them.

The manufacturer has done everything possible to guarantee that the equipment functions safely, both electrically and mechanically. The user must ensure that the instruments are set up and installed in such a way that their function is not impaired.

The instruments are tested by the manufacturer and supplied in a condition that allows safe and reliable operation.

This User's Manual includes information and warnings that must be observed by the user in order to ensure safe operation of the instruments.

Please adhere to the following safety instructions when handling or operating the system:

1. The instruments may only be operated by personnel who have been trained on the use of the systems. It is strongly recommended that all users read this manual prior to use.
2. Use the instruments only for the designated application.
3. BD Biosciences Pharmingen assumes no liability for any damages, including those to third parties, caused by improper use or handling of the instruments.
4. The user may only perform the maintenance work described in this manual.
5. Use only the parts described in this manual for servicing.

6. The instruments are live and improper handling may cause damage.
7. Before opening the instruments, disconnect the power supply.
8. Switch off the instruments before pulling the power cord.
9. Spare fuses must match the values specified by the instrument manufacturer. The fuses must not be short-circuited or tampered with.
10. All instruments supplied and all additional devices must be grounded. Use three-pole grounded plugs.
11. If you can see that the units have become unsafe to use, switch them off and disconnect them from the power supply.
12. If liquid gets inside the instruments, pull the power cord. Clean the unit or have it cleaned by an authorized service center.

The tests and maintenance work recommended by the manufacturer should be performed to make sure that the operator remain safe and that the instrument continues to function correctly.

Any service and maintenance work not described in the operating manual must be performed by authorized service engineers.

2. System Description

2.1 Overview

The **MONOLIGHT 3096 Microplate Luminometer** has been designed for the detection of chemi- and bioluminescence and for all measurements of glow and flash luminescence on microplates, i.e. for reactions where the light remain nearly constant over a longer period of time, as well as for very fast reactions requiring at least one injection during measurement. The **MONOLIGHT 3096** was designed to be used with the add-on injector unit for measurement requiring injection in the instrument. This unit allows injection of up to two substances in the instrument, even during measurement.

Highly sensitive light detector

The **MONOLIGHT 3096 Microplate Luminometer** is equipped with a highly sensitive light detector. A special measuring geometry ensures high sensitivity and a dynamic range covering more than 6 decades.

Low crosstalk

A special light guide system reduces crosstalk (light transfer between adjacent samples) to a minimum. Crosstalk can be reduced further by selecting suitable microplates.

Mechanical construction

The sophisticated mechanical construction of the instrument offers further advantages for the user:
The microplate is placed on a mobile transport unit outside the measurement chamber and moved into the measurement chamber for measurement by means of a highly precise control mechanism that positions the sample wells exactly below the photomultiplier. The separation of microplate loading compartment and measurement chamber ensures precise measurements, simple operation and little maintenance and cleaning.

Optional injector unit

The **MONOLIGHT 3096** is the basic unit which can be upgraded with the injector unit, containing 2 injectors. The **MONOLIGHT 3096** is designed for working with injectors (special devices for injector tips and tubings). In connection with the highly precise **injector unit**, measurements with up to two injectors can be performed.

Windows-based operation

Operation and control of the **MONOLIGHT 3096 Microplate Luminometer**, the injectors, as well as evaluation of the measured results take place via the Windows software **Simplicity Photon Counter** (abbreviated: **Simplicity PC**) which was specifically designed for this field of application. It is based on MS EXCEL and features clearly structured and intuitive user guidance.

Three measurement modes

Three measurement modes based on predefined measurement protocols are available:

Raw Data	Quick measurement of a microplate
Slow Kinetics	Kinetics measurement for longer light reactions. For each data point all samples are measured in succession.
Fast Kinetics	Kinetics measurement for short light reactions. The trend of the light emission of the 1st sample, then the 2nd sample etc. is measured.

The software is designed such that further measurement protocols can be integrated. This can be done by the user via EXCEL macros in the **Simplicity PC** software.
The **MONOLIGHT 3096 Injector Unit** works with the same PC software. Corresponding to the current instrument configuration, additional program functions are enabled when injectors are connected.

Fields of application

The **MONOLIGHT 3096 Microplate Luminometer** can be used for these, and other applications:

- Reporter gene assays
- Immunoassays
- ATP assays
- DNA and protein assays
- PCR quantification assays

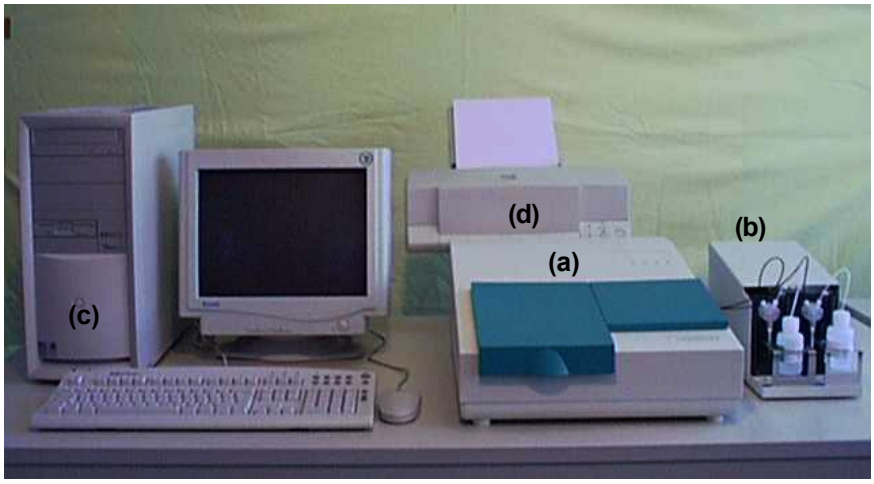


Figure 2-1: *Components of the measuring system: (a) MONOLIGHT 3096 Luminometer (b) Injector unit (c) PC (or laptop) (d) Printer*

2.2 MONOLIGHT 3096 Microplate Luminometer

The **MONOLIGHT 3096 Microplate Luminometer** is a compact, flat desktop unit with small footprint; due to its small size it can be set up on any lab workplace.

The **MONOLIGHT 3096** can work without or with up to 2 injectors. Simply connect the **MONOLIGHT 3096 injector unit** to the **MONOLIGHT 3096**.

CAUTION

To obtain reliable, consistent results, keep the following in mind:

- Do not expose instrument to direct sunlight or heavy temperature fluctuations.
- Set instrument up in dry rooms.
- Keep microplate loading compartment free of dust and dirt.
- Clean spilled reagents inside the instrument immediately using a clean and dry cloth.
- Open instrument door only for loading or cleaning to keep the inside dust-free.
- Open service door next to the instrument door only after having disconnected the instrument from main and when the instrument door is open!

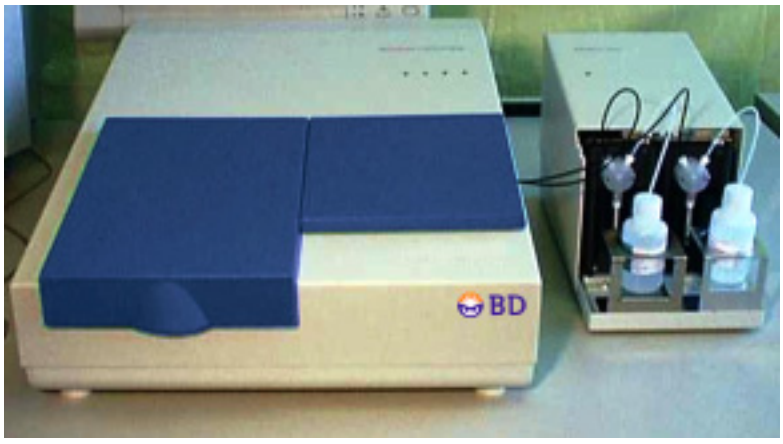


Figure 2-2
MONOLIGHT 3096 Microplate Luminometer with injector unit (front view)

2.2.1 Operating Components on the Front Panel

Four ***LED's*** inform you at one glance about the current status of the luminometer.

The LED's from right to left:

1.	**Green**	Microplate Luminometer is turned on and ready for operation.
2.	**Red**	Measurement is running. *Do not open instrument door!*
3. + 4.	**Yellow**	Data transfer between PC and luminometer

Instrument door

The instrument door for loading microplates is on the left instrument side. It is opened and closed by hand. When closing the door, make sure to push it into the spring lock. An audible click indicates that the instrument door is closed light-tight. If it is not closed correctly, a warning will appear on the display.

CAUTION

For correct measurements, the instrument door and the service door must be properly closed so that no outside light can enter the instrument.

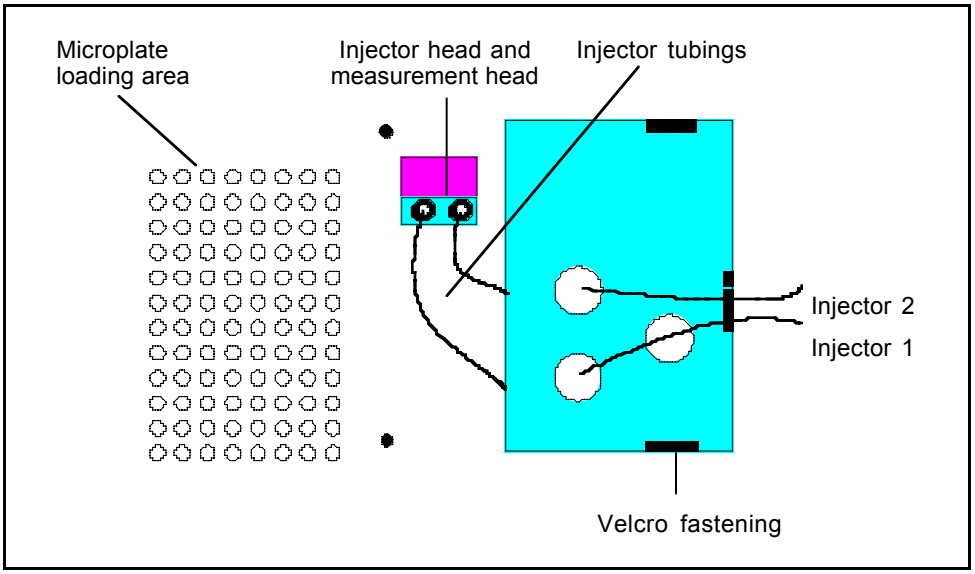


Figure 2-3: *MONOLIGHT 3096: View with instrument door and service door open*

Detachable service door

The service door is to the right of the instrument door. You will need this door only if you are connecting the **MONOLIGHT 3096 injector unit** (option) (see section 3.6).

2.2.2 Microplate Loading Compartment

The microplate loading compartment is a compartment that is separated from the measurement chamber; it is accessible via the instrument door.

In its home position, the mobile ***microplate transport unit*** is completely inside this compartment. After the power is turned on and upon completion of a measurement, the transport unit automatically moves to this position. This movement can also be controlled via software.

The transport unit is a mobile tray accommodating the microplate. Controlled via software, the transport unit can be moved into the measurement chamber so that the wells are exactly positioned below the photomultiplier one after the other. The transport unit can also be moved by hand. The transport unit comprises a ***spring-loaded support tray*** for the microplate with a device that prevents its slipping out of place and a ***frame*** which can be folded up.

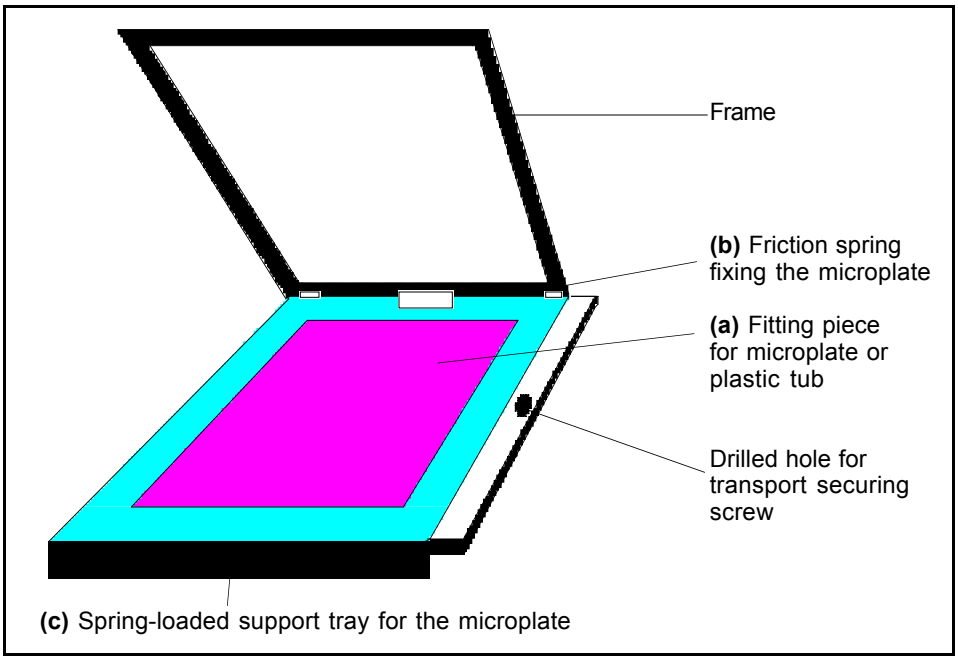


Figure 2-4: *Microplate transport unit with raised holding frame*

The ***spring system*** ensures that different microplate types, regardless of their height, will always be pushed directly against the frame and thus as close as possible against the photomultiplier. Since the sensitivity decreases with the squared distance to the detector, the spring system significantly increases the sensitivity of the measuring system.

If the MONOLIGHT 3096 is operated with injectors, a small plastic tray is supplied with the instrument. Put this plastic tray into the microplate loading compartment instead of the microplate when priming and washing the tubing to collect the liquid escaping from the injector tips.

Keep microplate loading compartment clean

- Keep frame and loading tray clean and free of dust. If necessary, clean it with a moist cloth. Dirt will also have an adverse effect on the measured result!
- If liquid does get *under* the microplate transport unit, you can move this unit by hand or via software into the measurement chamber, so that the bottom of the loading compartment can be cleaned.
- Always make sure that liquid does not get into the measurement chamber.

How to insert a microplate

❑ Make sure that the service door next to the instrument door is closed properly!

❑ Open the instrument door of the Microplate Luminometer.

❑ Turn up the frame of the transport unit. You cannot open the frame if the transport unit has been moved a bit to the right towards the measurement chamber. In this case, move it by hand all the way into the loading compartment.

❑ Place the prepared microplate onto the loading tray, such that the A1 well is in the upper, right-hand corner.

- ❏ Turn down the frame and push it shut until it snaps into place (audible click!). The microplate is held in place by the fitting piece (Figure 2-4: **(a)**), by the spring on the frame at the rear between the hinges **(b)** and by the spring-loaded support tray **(c)**.
- ❏ Close the instrument door. Push it shut until it clicks into place with an audible noise. Then, the instrument is closed light-tight.

2.2.3 Measurement Chamber

The measurement chamber is separated from the microplate loading compartment. It is sealed light-tight only when the service door has been closed properly.
For measurement, the transport unit, with the microplate, will move into the measurement chamber, so that the well to be measured is exactly positioned below the photomultiplier. The wells are measured column by column (A1 to H1, A2 to H2 ... A12 to H12).
For information on the position of the injector tips please read section 2.2.6.
The measurement chamber is not accessible to the user. Dirt in the measurement chamber can therefore be removed only by authorized service engineers.

Measuring range

2.2.4 Light Detector

The light detector measures visible light in the range from 300 to 650 nm. The photons emitted by the sample are converted into electrons and multiplied by a photomultiplier. The single pulses, which are digitally counted, are directly proportional to the emitted light quantity.

The light emitted by the samples can only be read from above and is – since the photomultiplier, for practical reasons (flat instrument design), is installed horizontally – reflected by a mirror by 90° nearly without any loss.

RLU In contrast to other physical units, luminescence is not indicated in fixed units of measure, for example, Lumen, but in "**Relative Light Units**" (RLU). Therefore, only results from the same luminometer type can be compared with each other. An RLU factor has been defined in order to compare results from different luminometers. This factor multiplies all results. Typically, the RLU factor is 1. Upon request, this RLU factor can be changed.

2.2.5 Background Measurement and Subtraction

The background is comprised of two components: instrument background and reagent background.
The reagent background is obtained by measuring reagents without any analyte. For example, with a reporter gene assays, this measurement is of the reagents measured without cell lysate.

2.2.6 Injector Compartment behind the Service Door

When your system includes injectors, the injector compartment of the MONOLIGHT 3096 located behind the service door next to the instrument door is equipped as follows:
The service door is located to the right next to the instrument door. On the right-hand side it is held by Velcro fastening. The plate covers the instrument area with the injector tubings, the injector tip holder and the measurement head (see Figure 2-5).

Caution: Since the photomultiplier is exposed to incident light when the service door is opened, you must disconnect the instrument from main before opening it to rule out damage to the photomultiplier. For measurement the service door and the instrument door must be closed properly! Never operate the instrument with the service door removed.

- Before opening the locking plate next to the instrument door, disconnect the instrument from wall socket and make sure the instrument door is open!
- First, open the 2 fastening screws of the locking plate and hold the plate at the screwed side and take it off! Otherwise, the locking plate may get damaged!

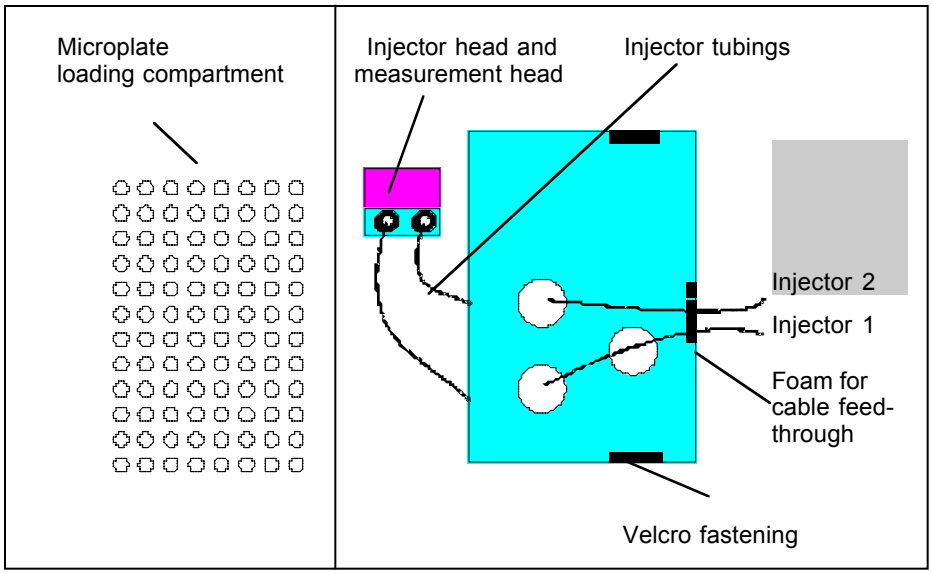


Figure 2-5: *MONOLIGHT 3096: View with open instrument door and service door*

The injectors are arranged as shown in Figure 2-6. The microplate moves from left to right. Injector 1 injects in the position before the measurement position, injector 2 into the well in the measurement position (see Figure 2-6).

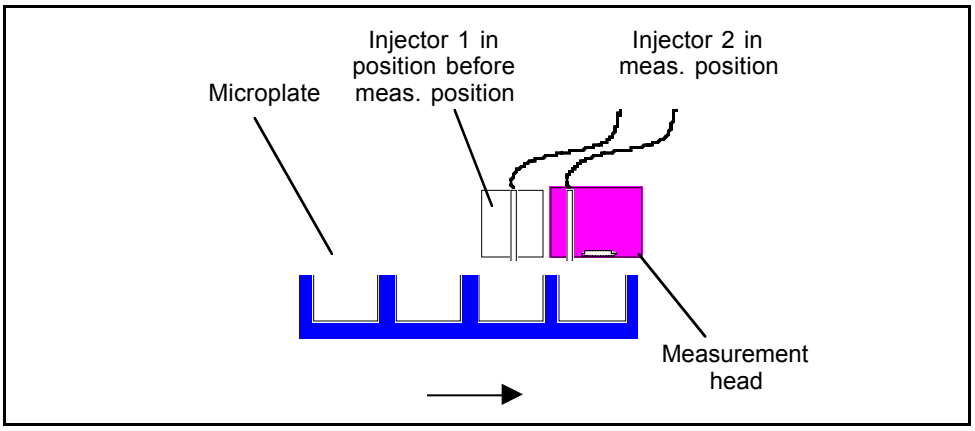


Figure 2-6: *Position of injectors above microplate*

You may take the injector head off to replace the tips and the injector tubings (see section 8, Maintenance).

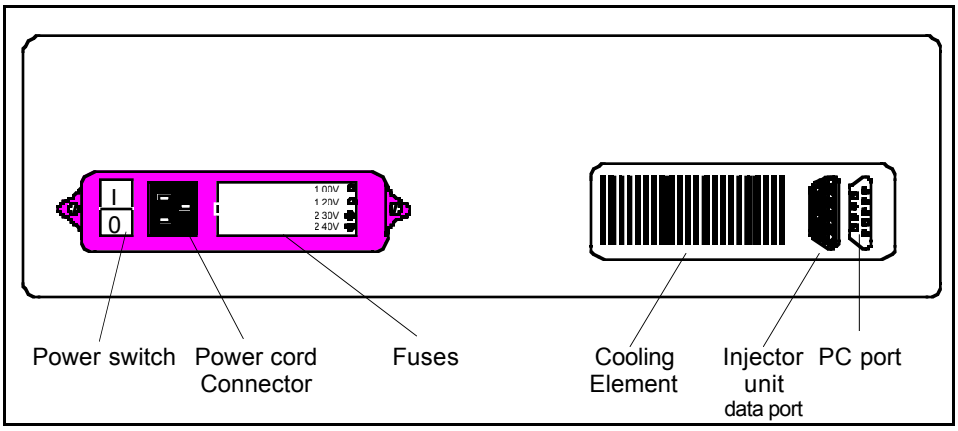
Closing the Service Door

- ❑ Close the service door carefully to ensure light cannot enter the measurement chamber.
- ❑ Put the tubing leading to the injector unit on the rubber foam, so that the door is pressed into the foam and is closed light-tight.
- ❑ Align the service door exactly.
- ❑ Then press the service door down firmly to close the Velcro fastening.

2.2.7 Connections on the Rear Panel

The Luminometer rear panel includes the connection ports, the power switch, the fuses and the cooling element.

Figure 2-7: *Rear panel of MONOLIGHT 3096 Luminometer*



Connect the **MONOLIGHT 3096** to the serial communication port of the PC using the supplied 9-pin data cable (with two female connectors).
Using the supplied cable (with two male connectors), connect the **MONOLIGHT 3096 injector unit** (optional) to the 9-pin socket of the **MONOLIGHT 3096** and to the 15-pin socket on the injector unit. Connect the instrument to wall socket via the supplied power cable.

CAUTION

Line voltage and operating voltage must always match!

CAUTION

Observe a minimum distance of 10 cm between the back of the instrument and the wall to allow air circulation for the cooling element!

Voltage change, see section 8.3.
Fuse replacement, see section 8.2.

2.3 Injector Unit (optional)

The **MONOLIGHT 3096 injector unit** consists of two highly precise Cavro pumps and the control unit which is integrated in the housing.
The injector unit is programmed such that the left pump is connected to the left injector tip in the MONOLIGHT 3096. In the **Simplicity PC** software the left injector is **Injector 1**.

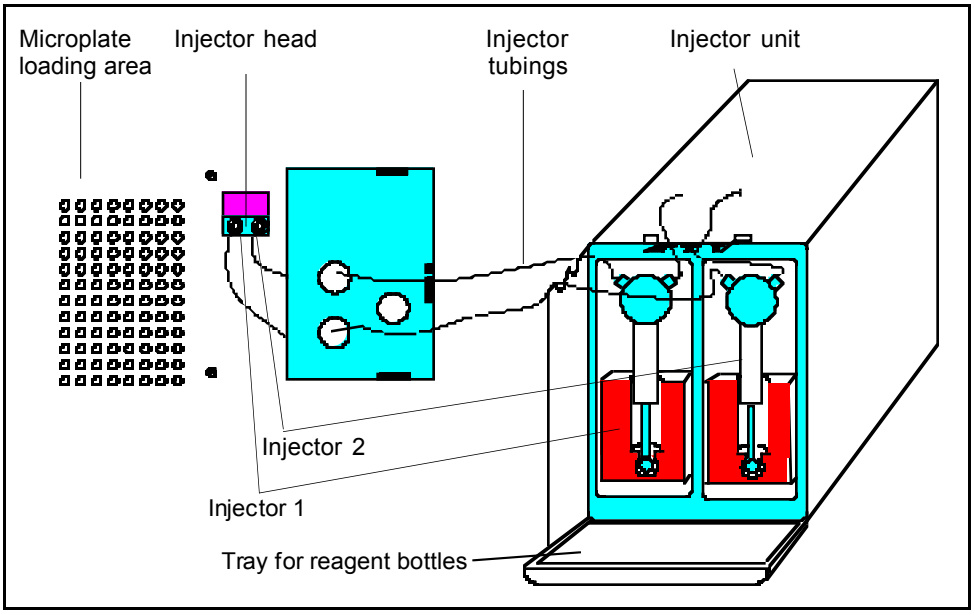


Figure 2-8: *MONOLIGHT 3096 with connected injector unit (both devices open)*

The right pump is connected to the right injector tip in the MONOLIGHT 3096. In the Simplicity PC software, the right injector is **injector 2**. The injector unit is closed by a **plastic cover** on the front which can be taken off to connect the tubing and to service the injector unit (see section 8). Lift the cover up vertically from above.

Pass the **injector tubings** left to the MONOLIGHT 3096 and up to the **reagent bottles** which are placed on the tray onto the front side of the injector unit. Pass the tubings through the respective **openings** and make sure you don't damage or squeeze them (see Figure 2-8).

The front side of the injector unit includes a detachable **tray** for the reagent bottles. On the rear panel you have access to the following ports:

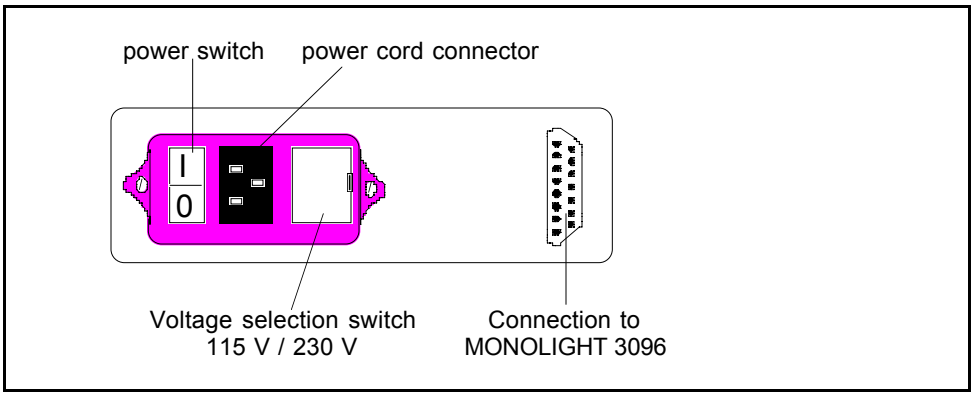


Figure 2-9: *Rear panel of injector unit with connection ports*

2.4 Microplates

All 96 well microplates in standard format can be measured on the **MONOLIGHT 3096 Microplate Luminometer**.

To obtain reliable, accurate results, you should only use ***non-transparent (opaque) microplates***.

Black microplates show the lowest crosstalk, but they absorb light, i.e. they are not suitable for samples with low light intensity.

White microplates are well suited since they reflect the light from the samples and thus increase the sensitivity, but may increase background.

For general use we recommend the Falcon® black opaque microplate, Cat. No. 01-05500.

2.5 PC

Minimum requirements:
- 486 PC or laptop
- minimum 16 MB RAM
- 200 MB hard disk
- 3.5“ disk drive
- Color graphics card
- Color monitor
- Keyboard and mouse

Any printer supported by Windows.

Software requirements:
- MS Windows 95
- MS EXCEL 7.0 (optional)

2.6 Software

The **MONOLIGHT 3096 Microplate Luminometer** is operated by the software package **Simplicity Photon Counter (Simplicity PC)** designed specifically for this instrument.

The program comprises three levels:
Protocol Manager: management of protocols and system parameters.
Measurement parameter input: depending on the selected measurement protocol.
Measurement menu: the measurement is performed as defined by the selected protocol type and the entered parameters and the results are displayed.

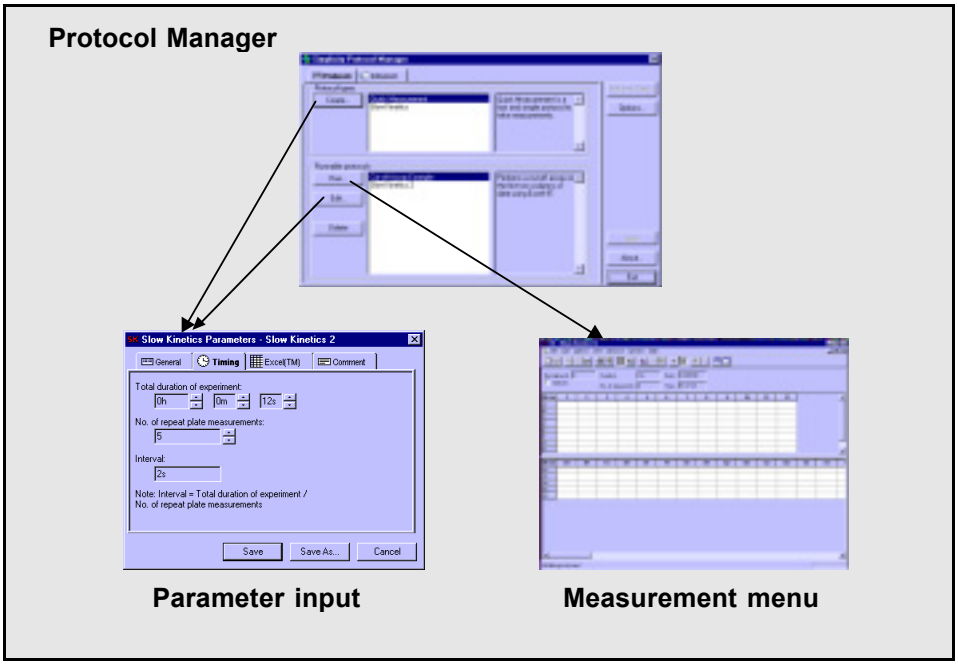


Figure 2-10: *Structure of* ***Simplicity PC*** *software*

3. Installation and Commissioning

3.1 Overview

Proceed as follows to prepare your first measurement:

3.2 Setup Site

The MONOLIGHT 3096 Microplate Luminometer must be set up in a dry, fairly dust-free room and protected from direct sunlight and significant temperature fluctuations.
Do not set it up next to a radiator.

3.3 Space Required

The luminometer is rather small (**MONOLIGHT 3096** W x D x H = 39 cm x 49 cm x 13.5 cm).

The instrument rear panel must always be at least 10 cm away from the wall or other devices to ensure that the cooling element can work properly and you have easy access to the power switch.

When using the injector unit (option) you must allow for an additional 20 cm (width).

3.4 Unpacking

Cardboard box

The luminometer is shipped in a cardboard box that contains two foamed inserts to protect the instrument against damage. It also includes a smaller box with cables (power cable, connection cable PC-Luminometer) and the software. The injector unit (option) is also delivered in a separate box. The cardboard box is reusable. It is recommended to store the box and packaging material for future shipping. However, boxes can be ordered separately. Luminometer packing material is Cat. No. 551484, and the Injector Unit packing material is Cat. No. is 551485.

Check shipment

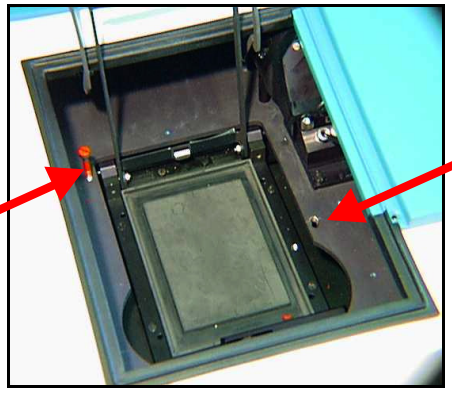
Unpack all units and accessories and ensure the shipment is complete and shows no sign of transport damage. The careful packing usually rules out transport damages. Should the instrument or instrument parts be damaged, please inform the shipping agent or the BD Biosciences Pharmingen immediately. Please be careful when unpacking the instrument and removing the foamed inserts.

3.5 Connecting the Instruments

- ❏ Carefully take the Microplate Luminometer out of the cardboard box and place it on your lab bench.
- ❏ Open the instrument door.
- ❏ Using a flat head screwdriver, unscrew the red transport securing screw, which holds the microplate transport unit (to the right next to the microplate frame).
- ❏ *Keep the screw and use it again to transport the instrument!* To the left of the sample compartment, there is a threaded hole in which the screw can be stored by screwing it in.

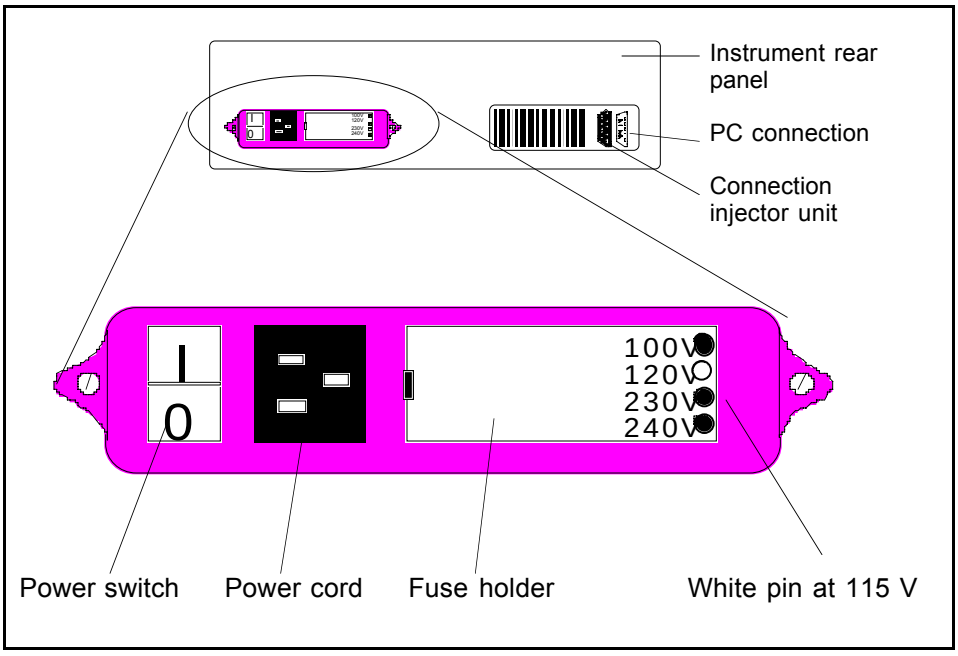
CAUTION: Save screw and install it again when transporting the instrument.

Figure: 3-1: *Microplate loading compartment with transport securing screw*



- ❏ Check if the wall socket voltage matches the operating voltage labeled on the instrument rear panel (label and position of the white pin on the fuse holder). If not, you must change the operating voltage by re-plugging the voltage selection card, so that the operating voltage matches local voltage. See section 8.3. Check and replace the fuses, if necessary. See section 8.2.

Figure 3-2: *Fuse holder with indication of operating voltage*



- ❏ Connect the luminometer and your PC or laptop to the serial communication port using the supplied connection cable which is provided with female connectors on both ends.
- ❏ When the wall socket and operating voltage match, connect the luminometer to the wall socket using the supplied cable.
- ❏ Turn the Microplate Luminometer on at the power switch (instrument rear panel). The green LED (1st from left) lights up and signals that the instrument is ready for operation.
- ❏ Perform a quick hardware test to check the microplate transport unit: open the instrument door and move the microplate transport unit by hand away from the home position. Now turn the instrument off and on again. After power is on, the microplate transport unit moves to the home position (audible and, with open the instrument door, visible movement). If the transport unit does not move to this position, please contact the Technical Service Department at 1-800-825-5832.
- ❏ If you have not done so yet: connect the computer to the printer and connect both devices to the wall socket.

3.6 Connecting the Injector Unit

The injector unit is supplied separately. Connect the injector unit to the **MONOLIGHT 3096** and then plug it in. A few simple steps suffice to connect the injector unit, since the injector tubings are already installed to the injector head of the luminometer and the other end of the tubes includes the pre-fabricated pump connection.

CAUTION

- ❑ First, unplug the MONOLIGHT 3096 Luminometer from wall socket.
- ❑ Put the injector unit to the right of the MONOLIGHT 3096 and pull the locking door of the injector unit off from above to expose the injector pumps.
- ❑ Open the instrument door of the MONOLIGHT 3096.
- ❑ Hold the service door on the left side and pull it off the Velcro fastening. You will find 2 rolled up injector tubings.

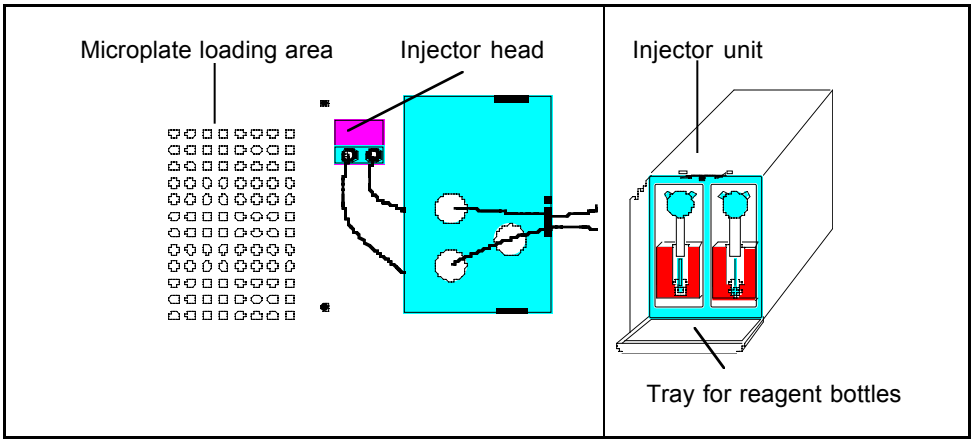
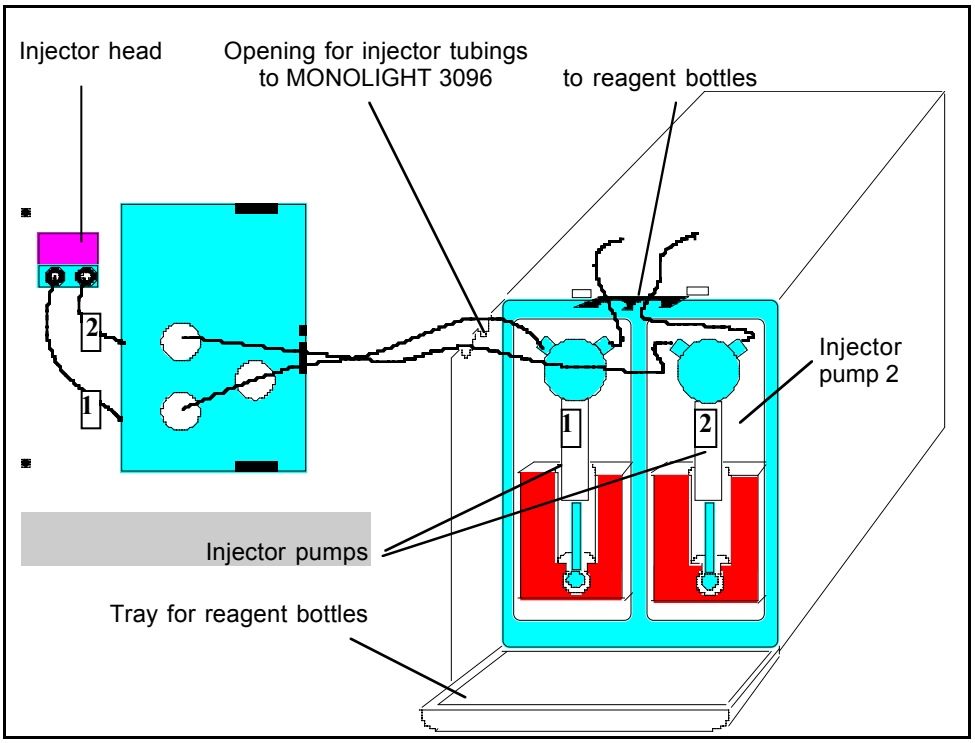
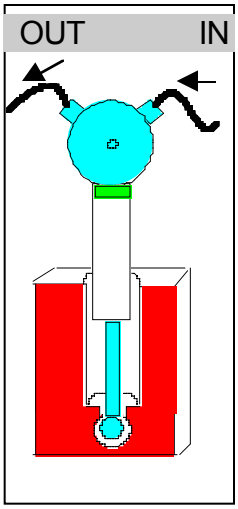


Figure 3-3: *Microplate loading compartment and injector (both devices open)*

❑ Pass injector tubing 1 to injector pump 1 and finger-tighten the fitting screw in the pump head (left connection = **OUT**). Proceed in the same manner with injector tubing 2. Pass the injector tubings at the MONOLIGHT 3096 over the rubber foam piece and at the injector unit through the openings on the side.

Figure 3-4: *MONOLIGHT 3096 with connected injector unit (both devices open)*





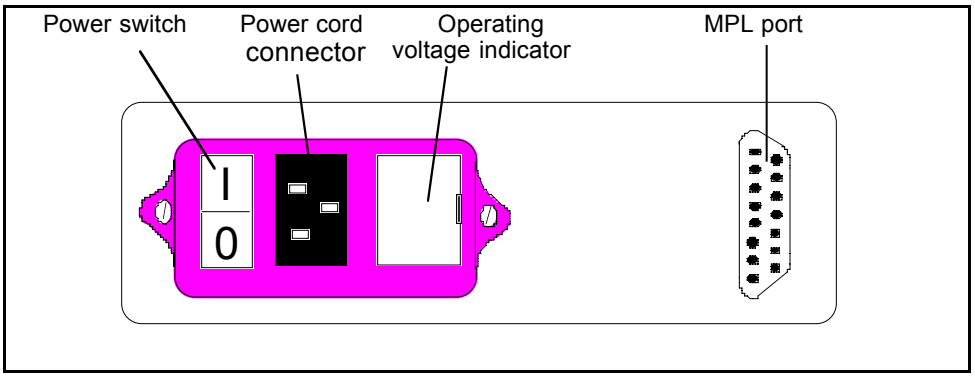
❑ Cut the tubings connected to the **IN** ports (right) of the pumps to the length you need and connect them to your reagent bottles. Make sure the tubings are long enough to pass them through the openings on top of injector unit and to the bottom of the reagent bottles standing on the tray attached to the front of the injector unit.

❑ Close the injector unit again by inserting the locking door vertically from above. Pass both slots on the bottom side of the door through the openings at the bottom of the injector unit, the two slots on top of the injector unit through the openings on the door.

❑ Close the service door at the **MONOLIGHT 3096** very carefully to prevent any light from entering the measurement chamber:

- ❑ The tubings leading to the injector unit must be installed at the foreseen place (rubber foam) so that the door is pressed into the rubber foam.
- ❑ Align the service door exactly.
- ❑ Press the service door down firmly on the right hand side.
- ❑ Connect the MONOLIGHT 3096 and the injector unit using the supplied cable. Insert the 15-pin connector into the MPL port in the injector unit.

Figure 3-5: *Rear panel of injector unit*



CAUTION

- ❑ **Check that the wall outlet voltage matches the operating voltage labeled on the injector unit. If not, change the operating voltage as described in section 8.14.**
- ❑ Connect the injector unit to the wall outlet using the supplied power cable.
- ❑ **CAUTION:** Always turn the luminometer on first, then wait for at least 20 seconds before turning on the injector unit.
- ❑ To stabilize reagent bottles, you may use the supplied reagent bottle holders made of stainless steel.

3.7 Software Installation

The **Simplicity** software includes an automatic installation program. The Simplicity software is compatible only with PC format, which must have Windows 95 or better installed.

See page iii for Typographical Conventions.

Installation of Simplicity PC:

- ❏ Close all Windows applications.
- ❏ Insert program disk in drive **A:**
- ❏ Select the Windows 95 command [**Run...**]. The [**Run**] dialog box appears.
- ❏ Type **A:\spcsetup**. This will start the setup program and display the start screen.
- ❏ Click <**Next**>. The [**Select Destination Directory**] dialog box appears and you can select the destination directory and the directory **C:\Program Files\Simplicity-PC** (Figure 3-6).
- ❏ Click <**Next**> if you want to keep the defaulted directory.

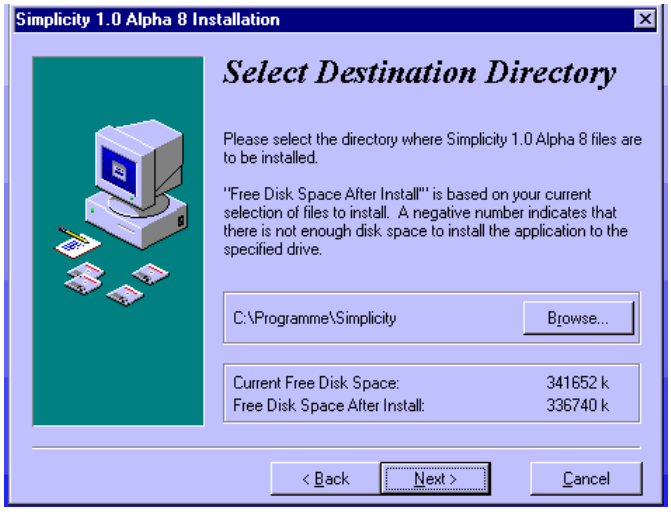


Figure 3-6: *Selection of destination directory*

Click <**Browse**> if you want to choose another directory. The [**Select Destination Directory**] dialog box appears. In the [**Path**:] text box, type the name of the new directory. Confirm your entry with <**OK**>. The new directory is created and the program returns to the [**Select Destination Directory**] dialog box.

❑ Click <**Next**>. The [**Ready to Install**] dialog box is displayed (Figure 3-7).

❑ Click <**Next**> to install the program. Upon successful completion of the installation process, you will see a corresponding message.

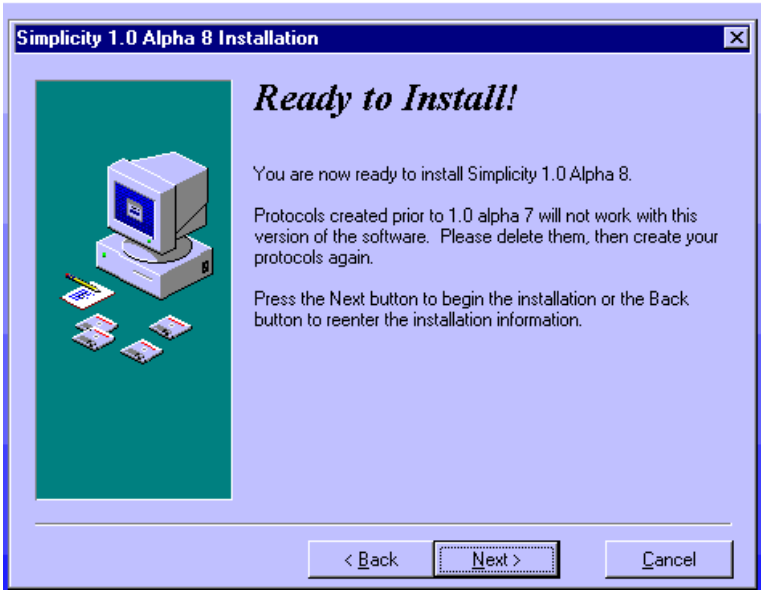
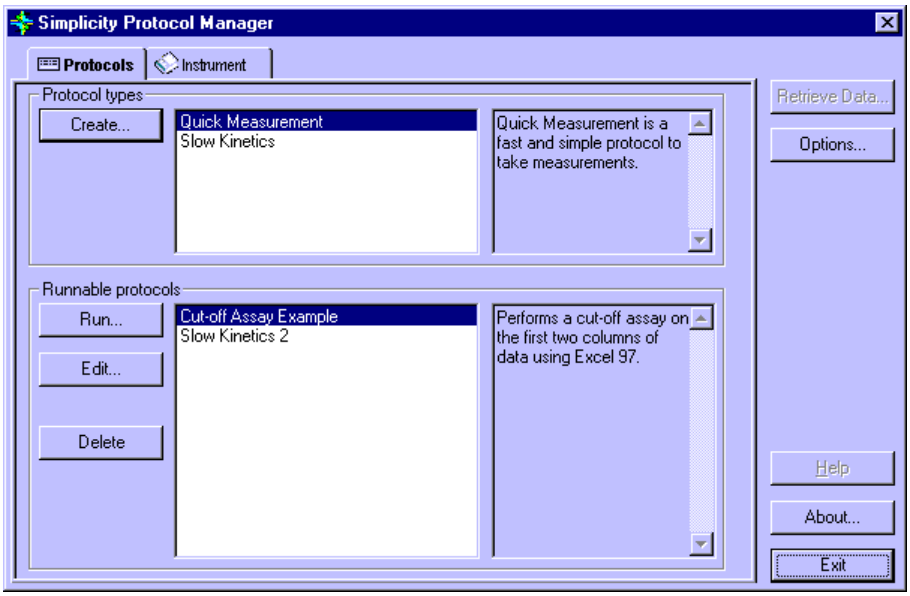


Figure 3-7: ***[Ready to Install]*** *dialog box*

3.8 Program Start

To start the **Simplicity PC** program, click the Windows 95 <**Start**> button, then select [**Programs**] and [**Simplicity PC**].
Simplicity PC is loaded and the **Protocol Manager** displayed with two tabs (see Figure 3-8).

Figure 3-8: *Simplicity Protocol Manager*



The program is configured such that the instrument is initialized only upon selection of the measurement menu (<**Run**>). In this manner you can first define the communication port used.
You can change this presetting in the [**Instrument Options**] dialog box, so that the initialization takes place when the program is started (see section 3.9 Instrument Configuration). In this case, communication with the luminometer is checked when the program is loaded. When the communication has been established, the program is fully operational. Otherwise, you cannot call the measurement menu.

3.9 Instrument Configuration

For instrument configuration, select the [**Instrument**] tab in **Protocol Manager** and make the following settings (see Figure 3-9).

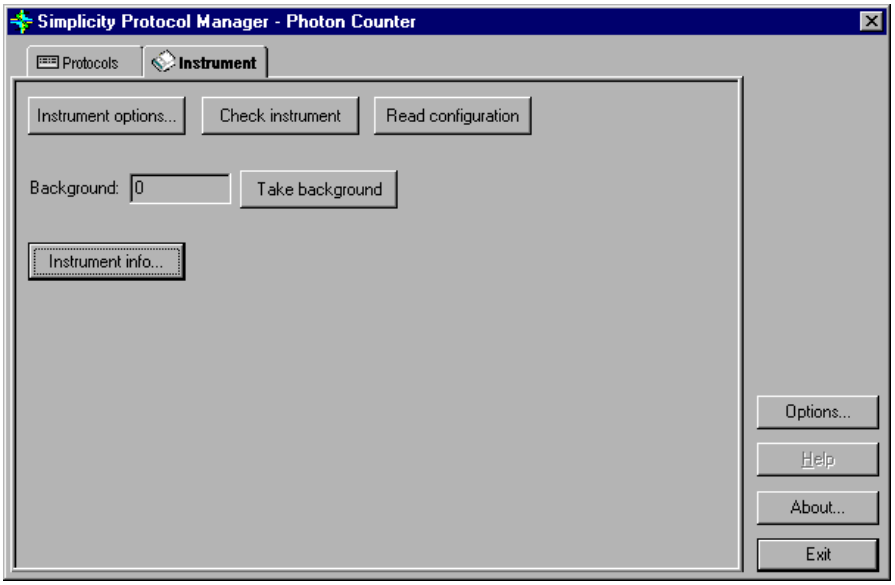


Figure 3-9: *[**Instrument**] tab*

❑ Click <**Instrument options...**>. The [**Instrument Options**] dialog box appears (Figure 3-10).

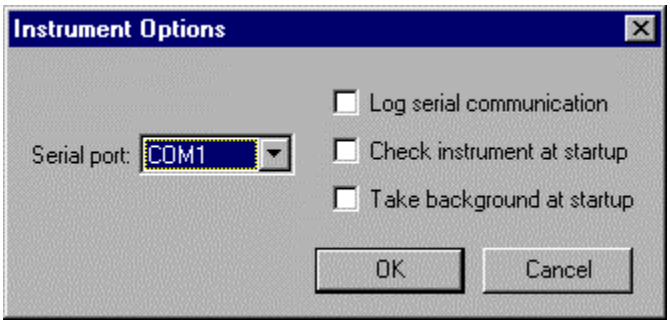


Figure 3-10: *[**Instrument Options**] dialog box*

❑ Select the communication port used in the field [**Serial Port**] (**COM1** or **COM2**). In most cases, **COM 1** is the proper choice, rarely **COM 2**. If you select the wrong communication port, the error message **"The instrument does not respond**" is displayed. In this case you must select the other port.

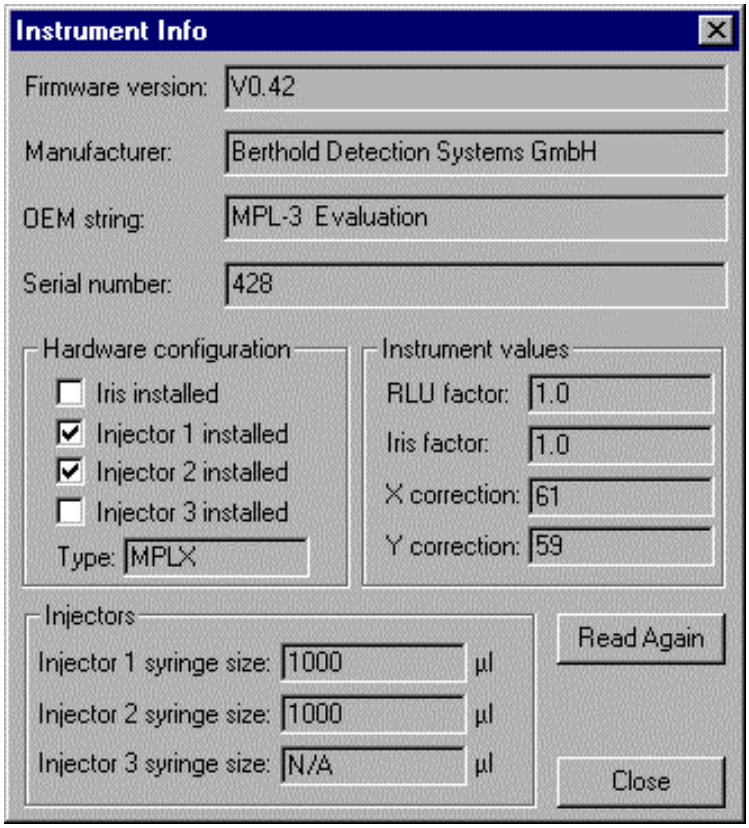
- ❑ [**Check instrument at startup**]: Select this item if you want to initialize the Microplate Luminometer automatically any time the program is started. Otherwise, an initialization is performed only after you select the measurement menu or click the <**Check instrument**> button on this tab. Upon delivery of the software, this item is not enabled.
- ❑ [**Take background at startup**]: Select this item if you want to run a background measurement any time the program is started.
- ❑ Confirm your entries by clicking <**OK>**. The [**Instrument Options**] dialog box is closed, the [**Instrument**] tab is active (Figure 3-9).
- ❑ Click <**Check instrument**> to establish communication between PC and luminometer. If this is not possible, an error message appears and you are prompted to check the connections and the communication port parameters. If an initialization is performed this is indicated on the status bar and by the yellow LED lighting up on the luminometer, while the instrument modules are being checked.
- ❑ Click <**Read configuration**>. Then the current instrument configuration (with or without injector(s) is read in from the **Simplicity PC** program.

You may carry out the following steps, if required:

- ❑ To run a background measurement, remove any light source from the measurement chamber, close the loading cover and click <**Take Background**>. While the background measurement is on, the red LED lights up on the luminometer. The measured background value is displayed and remain stored until the next background measurement is performed. It is also displayed in the measurement menu. Select [**Subtract**], to subtract this value from the measured sample values.

❑ Click on <**Instrument Info**> on the [**Instrument**] tab to view the instrument configuration (Figure 3-11). The current instrument parameters are displayed, such as number and size of the installed injector. The parameters cannot be changed. Please contact **BD Biosciences Pharmingen** if you want to change the RLU factor.

Figure 3-11: [Instrument Info] *dialog box*

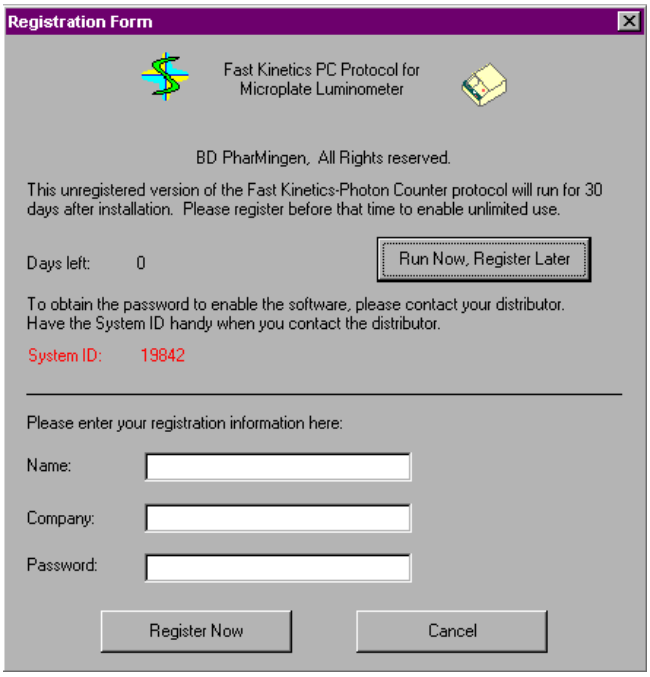


❑ To define the subdirectory to which the measured data are to be saved automatically, click <**Options**> on the [**Instruments**] tab and then enter the respective path name.

3.10 Software Registration

The **Simplicity PC** software can be used for 30 days without registration (after initial installation). During this time you will be prompted to enter a registration password any time you call a protocol or go to the measurement menu (see Figure 3-12). During this time, you can close the [**Registration Form**] dialog box by clicking <**Run Now, Register Later**> and then continue working with the protocol.

Figure 3-12: [Registration Form] dialog box



If you did not enter a correct password after 30 days, you cannot call a protocol anymore. The [**Registration Form**] dialog box appears again and you either have to enter your registration password or quit this dialog box with <**Cancel**>.

Registration

To get your registration password, contact **BD Biosciences Pharmingen, Technical Services, 1-800-825-5832**.

If the [**Registration Form**] dialog box appears when you call a protocol, enter your name, the company name and your password. Then click <**Register Now**>. If your password entry is correct, you can continue working with the software. The [**Registration Form**] dialog box does not come up any more.

After registration you may uninstall the program (using the command [**Uninstall**]) and install an updated version without having to enter a registration password.

3.11 First Measurement with Raw Data

Example of Measurement without Injectors
Since the detector needs some time before reaching its operating temperature, the results measured just after power on may differ slightly from those measured after the detector has reached its operating temperature.

Therefore, we recommend that you
- **start the instrument at least half an hour before running the first measurement**
- **leave the instrument turned on between individual measurements.**

❑ Open the instrument door.

❑ Turn up the frame of the microplate support tray.

❑ Place the prepared microplate on the support tray so that it sits correctly in the fitting piece and the letters are in the back. Well A1 should be in the upper right hand corner.

❑ Turn the frame down and push it shut until it snaps into place.

❑ Check that the microplate sits correctly.

❑ Close the instrument door and push it shut until it clicks into place.

❑ If you have not done so yet: Load the **Simplicity** software (See Section 3.7). Then the **Protocol Manager** appears.

❑ In the field [**Protocol type**], select the protocol type [**Raw Data PC**] and click <**Create**>. The [**Raw Data PC Parameters**] dialog box appears with several tabs for input of the measurement parameters.

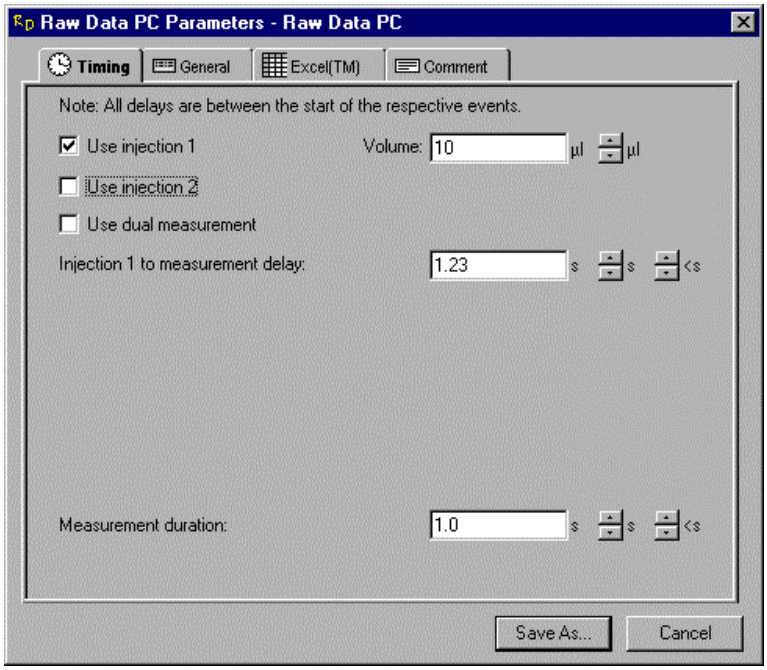


Figure 3-13: *[**Timing**] tab in the [**Raw Data PC Parameters**] dialog box*

❑ First, the [**Timing**] tab appears.

❑ When only one injector is used, select either, [**Use injection 1**], or [**Use injection 2**]; if your system includes the Injector Unit.

❑ Enter required measuring time per well. The default value is 1 second.

❑ Select the [**General**] tab and check the microplate type you are using (96 wells).

❑ Select [**Take background at start of plate measurement**] if you want to run a background measurement prior to the sample measurement.

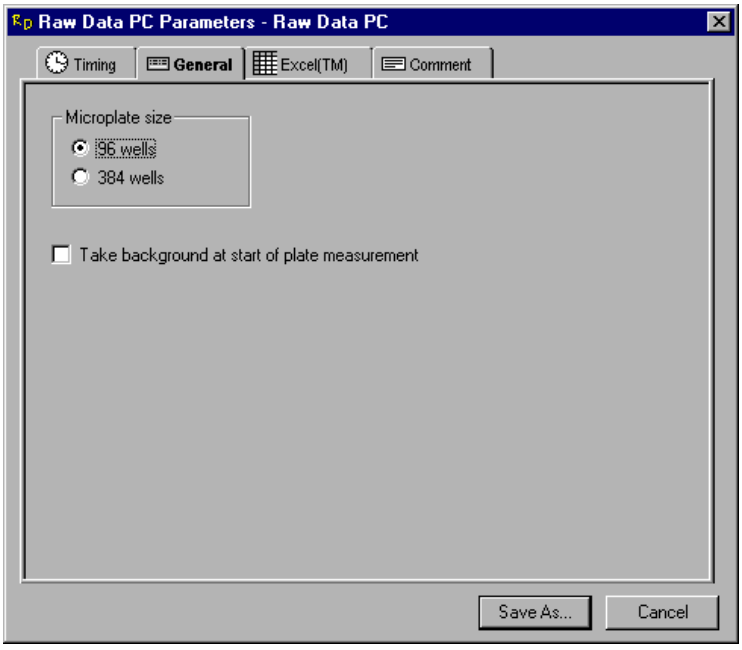
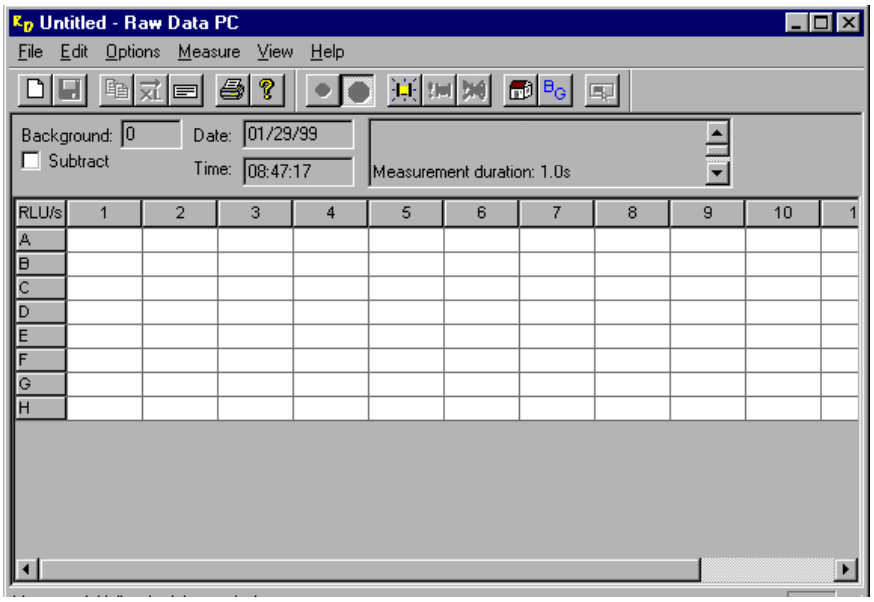


Figure 3-14: *[**General**] tab in the [**Raw data PC Parameters**] dialog box*

- ❑ The [**EXCEL (TM)**] tab is not needed for the first measurements.
- ❑ Select the [**Comment**] tab if you want to enter a comment for the measurement protocol. It appears after the protocol has been stored in the **Protocol Manager** next to the measurement protocol list when the respective measurement protocol is selected.
- ❑ Click <**Save As...**> and enter a file name in the following dialog box. It must differ by at least one character from the file name of the selected protocol type.
- ❑ Confirm the file name with <**OK**>. The program returns to the **Protocol Manager** and shows the name of the new measurement protocols in the field [**Runable protocols**].
- ❑ In the field [**Runable protocols**] in the **Protocol Manager**, select the new measurement protocol and click <**Run**>. Then the measurement menu is displayed (Figure 3-15).

Figure 3-15:
[Raw Data PC]
Measurement menu



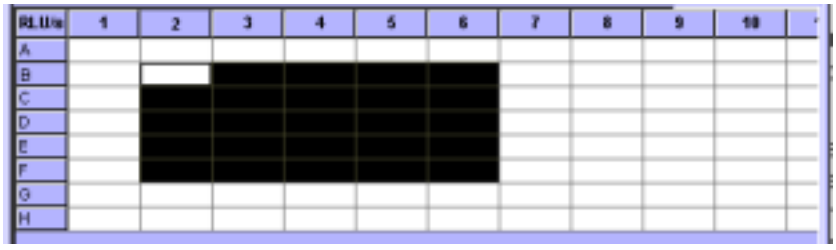
Measuring the entire microplate

❑ Click the  button.

Measuring a selected area

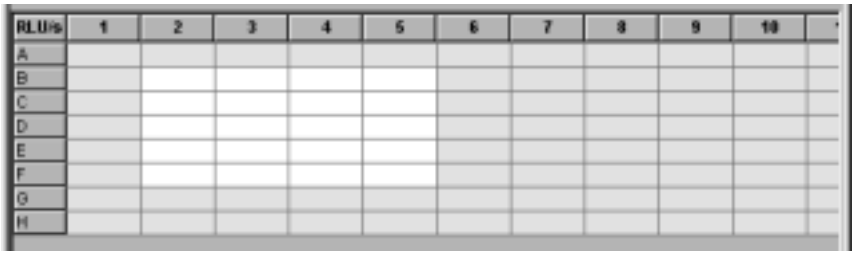
❑ Click on the first well of the area you want to measure and with the mouse button held down, drag the cursor over the wells to be measured. These wells are highlighted by a dark color (Figure 3-16). Release the mouse button when the desired area is marked.

Figure 3-16:
Selected well area



❑ Click the  button. Then the selected wells turn white, the others are gray-shaded (Figure 3-17).

Figure 3-17:
Defined well area



❏ The marked area is cleared again when you define a new area with the mouse and click the  button again.

Running a measurement

❏ Click  to start the measurement. The defined measurement procedure begins: the individual wells are selected in succession and measured using the predefined measurement time. The results are displayed in the well matrix on the screen. These are net results, provided a background measurement has been carried out prior to the measurement (display of the BG values in the field [**Background**]) and the item [**Subtract**] has been activated.

❏ Click  to enter a comment for the measurement which will be printed out in the report. This opens the [**Measurement Comment**] dialog box and you can enter your text.

❏ Click  to save the measurement data and then enter a file name.

Next measurement

- ❏ *To run the next measurement with the same measurement protocol*, click  (New measurement). The next measurement can be started immediately when the data of the preceding measurement have already been saved. Otherwise you will be prompted to save this data. If you have stored the data or if you have replied <**No**>, the screen is cleared for the next measurement and the icon buttons are enabled again.
- ❏ *To run the next measurement with another measurement protocol,* select [**Exit**] on the [**File**] menu. If the data of the preceding measurement have not yet been stored you will be prompted to save this data. After you have stored the data or if you have replied <**No**>, the program returns to the ***Protocol Manager*** and you can select a new measurement protocol.

4. Structure of the Software

4.1 Software Structure

The **Simplicity** software comprises three levels:

1. The **Protocol Manager** which is displayed after program startup is the program's switchboard featuring the following functions:
   - Create measurement protocols: <**Create**...>
   - Edit measurement protocols: <**Edit...**>
   - Delete measurement protocols: <**Delete**>
   - Select measurement menu: <**Run...**>
   - Load data file: <**Retrieve Data**>
   - Define instrument parameters: [**Instrument**] tab
   - Define directory for measured data: <**Options**>
     (see section 4.2)

2. **Input of measurement parameters**. To enter measurement parameters, either create a new measurement protocol in the protocol manager (<**Create...**>) or edit an existing measurement protocol (<**Edit...**>). The respective protocol is loaded and can be filled out and saved for later measurements (see section 4.3).

3. The **measurement menu** is structured depending on the selected protocol type. Here, the measurement is started and executed. At the same time, the measurement menu is the switchboard for display and further processing of the measured results: result display, graphical presentation and export of the data into EXCEL (see section 4.4).

Section 4.6 includes an overview of the program functions.

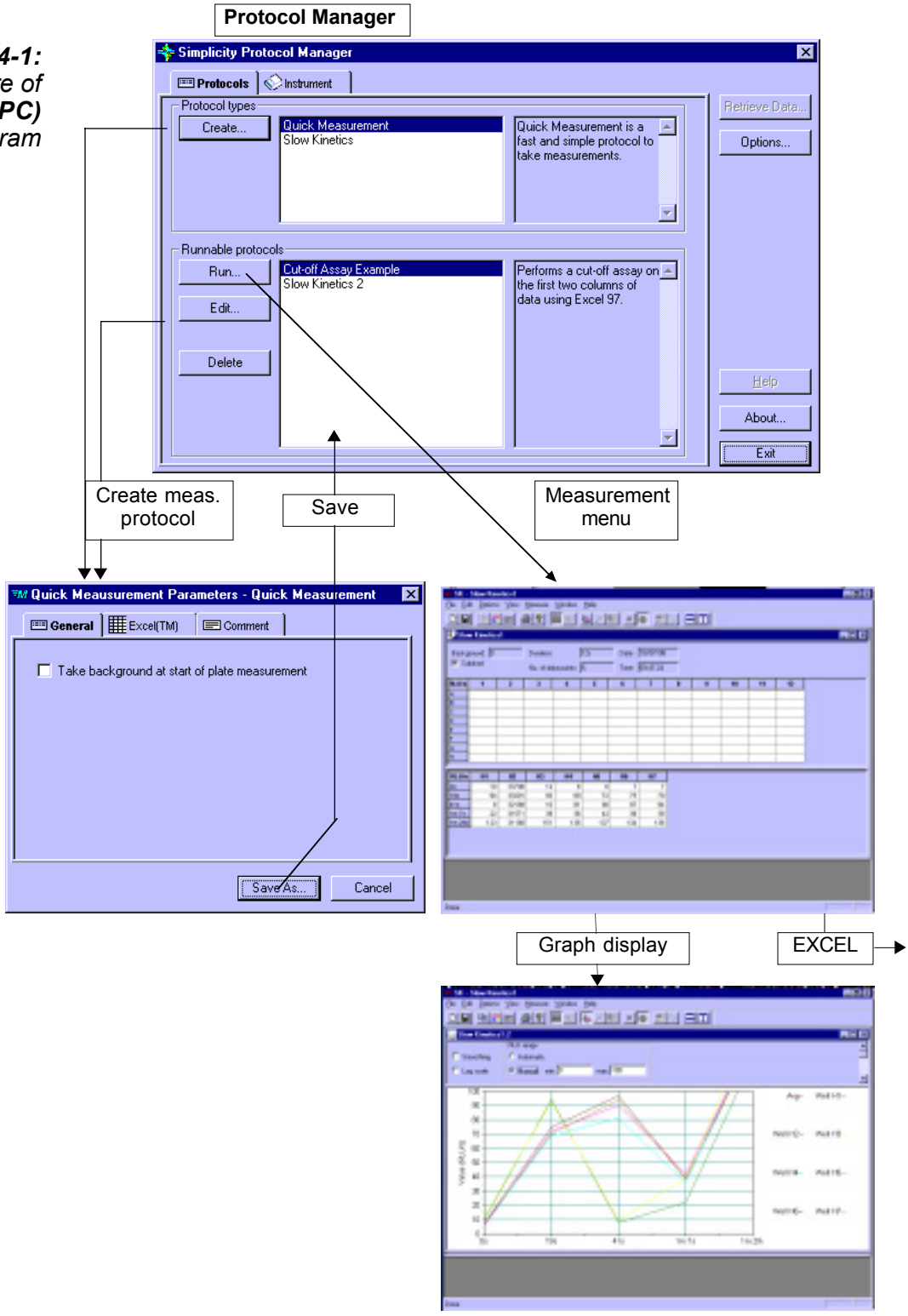


Figure 4-1: *Structure of* ***Simplicity (PC)*** *program*

4.2 The Protocol Manager

After startup of the software, the **Protocol Manager** appears and you can create and edit protocols and start measurements:

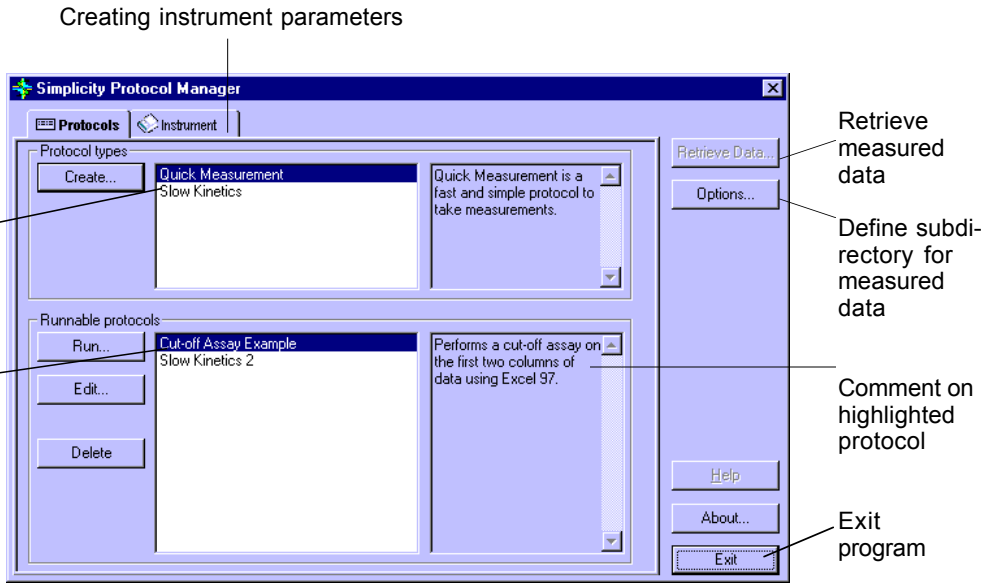


Figure 4-2: *Simplicity Protocol Manager*

4.2.1 [Protocols] Tab

[**Protocol types**]

The implemented protocol types are displayed in this field. Each protocol type includes a group of parameters for a special measurement method such as the *measurement time*, but *without any values*. The name of a protocol type therefore refers to the measurement method for which it is used, for example [**Slow Kinetics**] for kinetics measurements with light emission over a longer period of time. A protocol type always serves as *template* for a measurement protocol which includes certain parameters defined by the user. Based on one protocol type, you can set up as many measurement protocols with different parameters desired.

To create a measurement protocol, click <**Create**>, i.e., select a protocol type as template, enter the required parameters and save it under a new name. The program stores measurement protocols automatically in the field [**Runable protocols**] and lists them in alphabetical order. The name of a measurement

protocol must not be identical with the name of the protocol type, but should refer to the measurement method. You can enter a comment for each measurement protocol; it appears in the field next to the measurement protocol list, when the respective protocol has been selected (see Figure 4-2).

[**Runable protocols**]

The available measurement protocols are listed in alphabetical order. They are based on a selected protocol type and include the parameters defined by the user. The following buttons are available to edit measurement protocols:

<**Run...**> Opens the measurement menus to start a measurement with the selected protocol.

<**Edit...**> Shows the selected measurement protocol and lets you change it.

<**Delete**> Deletes the selected protocol.

4.2.2 [Instrument] Tab

On this page, enter and view the instrument parameters and initialize the instrument. Only the buttons <**Instrument options...**>, <**Check instrument**> and <**Take Background**> are important for the user.
<**Instrument options...**> takes you to the [**Instrument Options**] dialog box to define the instrument communication port (see section 3.9 Instrument Configuration).

Further Protocol Manager Options

<**Retrieve Data**> Load and display stored measurement data.

<**Options**> Select subdirectory to which the measured data are to be saved.

<**Exit**> Exit program.

4.3 Parameter Input

Select a protocol type and click <**Create**> or select a measurement protocol and click <**Edit**> to display the respective measurement protocol for parameter input (Figure 4-3). The title bar shows the file name of the protocol. This dialog box comprises several tabs. To open a tab, simply click on it.

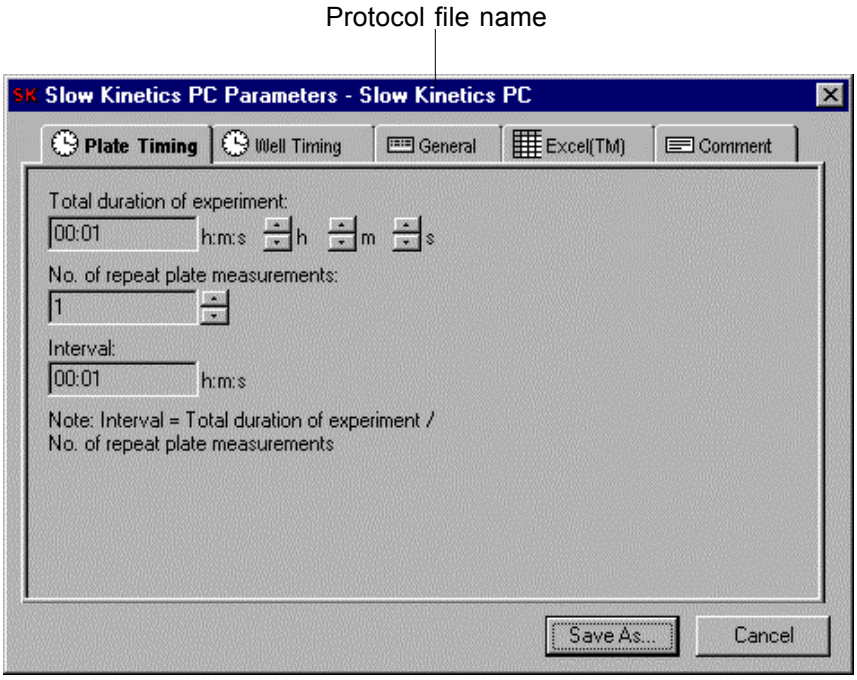


***Figure 4-3**: Measurement protocol [**Slow Kinetics Parameters**], [**Plate Timing**] tab*

As soon as you have entered your parameters, click <**Save As...**> to save the protocol under a new name or click <**Save**> to save it under the old name. The stored measurement protocol is then filed in the field [**Runable protocols**].
A new measurement protocol does not include the <**Save**> button; the file still bears the name of the protocol type which may not be used for saving this protocol.

4.4 Measurement Menu

Upon selection of the measurement protocols in the **Protocol Manager** and clicking on <**Run...**>, the program displays the measurement menu which is structured according to the selected measurement method.
At this point the luminometer is initialized, if this has not been done yet (see section 3.9).

4.4.1 Structure of the Measurement Menu

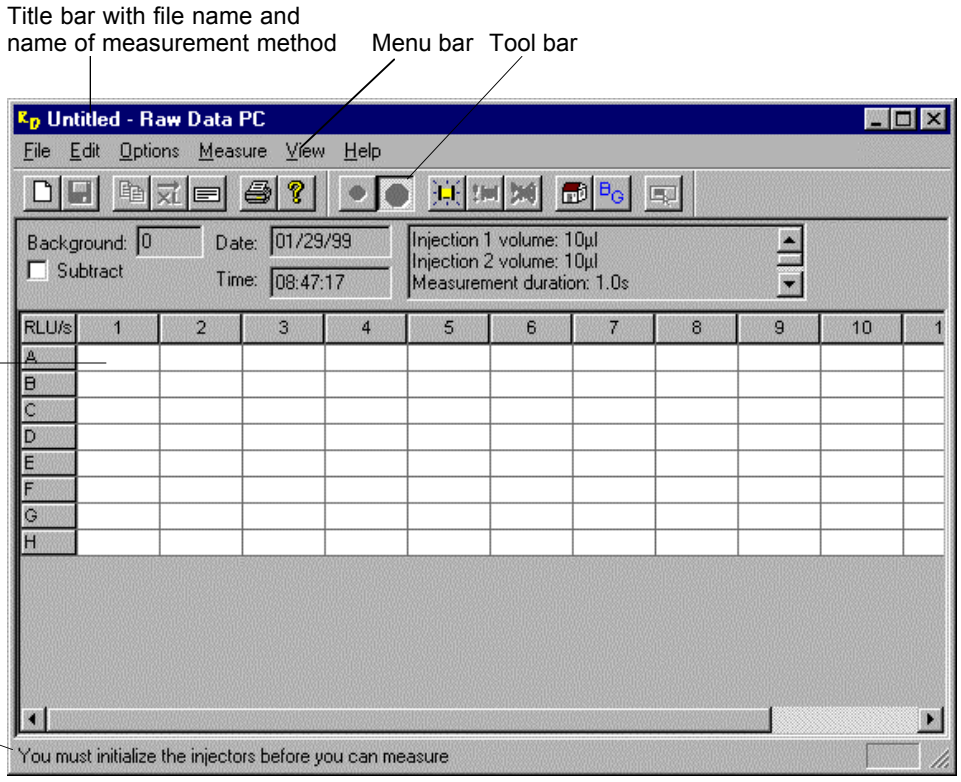


***Figure 4-4**: Measurement menu [Raw Data PC]*

Title bar shows the measurement method.

Menu bar shows the titles of the pulldown menus.

Tool bar shows the tool buttons you can work with in the displayed measurement mode.

Measurement Parameters The date, time, background, injectors used and the measurement time are displayed below the toolbar. You can choose if the displayed background value is to be subtracted from the measured values.

Sample matrix The microplate is depicted as a sample matrix with each well being represented by a box. Here you can select the wells to be measured. For **Raw Data**, the measured results are entered in the respective boxes, for **Slow Kinetics** and **Fast Kinetics** measurements are displayed in a separate table.

Status bar The status bar keeps you informed about the status of the sample drawer and the next operating steps in accordance with the selected measurement protocol.

4.4.2 Working with the Measurement Menu

This section briefly describes all functions that all measurement methods have in common. The functions which all measurements with injectors have in common (for example, priming) will be discussed at the end of this section.

All important functions in the measurement menu can be selected via tool buttons. Buttons or menu items that cannot be selected are dimmed. Some buttons are dimmed (gray), for example, when a measurement is running or no contact has been established with the luminometer or the data of the last measurement have not yet been saved. In all these cases a measurement cannot be started.

Autosave Function

Upon selection of this function, the results are automatically saved to files. The file names comprise an abbreviation of the measurement method (RD, SK and FK) and a consecutive number. This ensures fast storage of the measured data according to the selected methods.
Before starting a measurement, first select the [**Autosave**] function on the [**Options**] menu (✔= active). It will remain active until you deselect it again.

Selection of the sample area to be measured (see also page 39)

All wells of the microplate
As default, all wells of a microplate are selected for the following measurement. When you click the Start button  , the entire microplate is measured.
Selecting partial areas
If you want to measure one segment of the microplate, click the  button, mark the desired area with the cursor. Then click the  button again to confirm the selection and to exit the select mode (see section 4.4). The wells selected for measurement are depicted white on the screen, the others shaded gray.

Background measurement

Before starting a measurement, you can run a background measurement by pressing the BG button. The result is displayed in the [**Background**] field. Select [**Subtract**] if you want to subtract the background of the individual sample values.

Measurement start

Measurement of the microplate or the selected area starts after pressing the Start button (green signal light), when the instrument door is closed. The red LED lights up on the instrument.

The Start button is dimmed when a measurement is running, when there is no contact with the luminometer or when the data of the last measurement have not yet been saved. In all these cases, a measurement cannot be started.

If the instrument door is not closed, a warning appears informing you about the current measurement mode:

Close the instrument door and click <**Repeat**> or <**OK**>. Then the measurement starts.

Measurement procedure

The measurement procedure varies depending on the selected measurement method.

Measurement end

The measurement is over when all selected wells have been measured. In the autosave mode the measured results are stored automatically and the measured values cleared from the screen. If the autosave mode is not active the measured values remain displayed and can be stored later.

Measurement stop

Press the Stop button (red signal light) to stop the measurement. The results measured up to now can be stored.

Comment To enter a comment for the measurement, click the Comment button. The text entered in the [**Measurement Comment**] dialog box is printed in the report and saved to the data file.

Save You can save the measured results under any name by clicking the Save button.

If you want to quit the measurement menu without saving the data or if you click the button for new measurement  , you will be prompted to save the data.

In the autosave mode, each measurement is stored automatically and the file name (RD, SK or FK) is provided with a consecutive number.

New measurement

a) When using the same measurement protocol:

In the *autosave mode* you can immediately start a new measurement as soon as a measurement is finished. You can keep the selected area or define a new area as describe above.

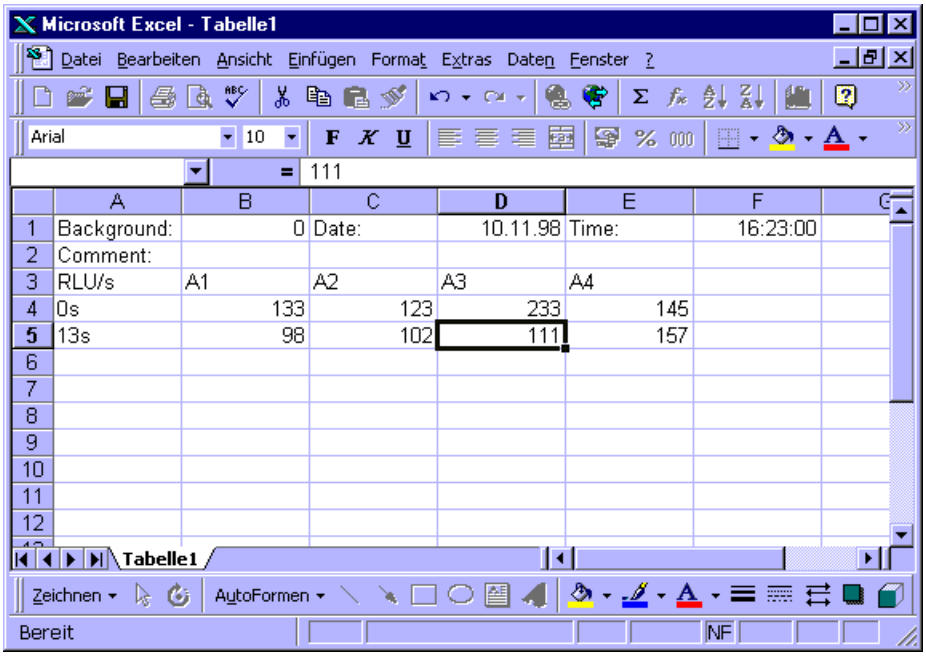
In the *normal save mode*, the measurement is cleared for the next measurement when you click on the  button (= new measurement), if the measured data of the last measurement have already been saved. Otherwise you may continue only after you have either saved the data or confirmed that you do not want to save the data. Clearing means that the measured data of the previous measurement are cleared from the screen and all tool buttons are active again. You may keep the selected area or define a new one as describe above.

b) When using a new measurement protocol:

To load a new protocol you must to exit the measurement menu via [**File**] / [**Exit**]. The program changes to the **Protocol Manager** and you can select another measurement protocol and start with <**Run**>.

Excel Transfer

To transfer the measured data to **Excel**, click the  button. **Excel** is started – provided it is installed on your PC - and the measured data are imported into an Excel spreadsheet.



	A	B	C	D	E	F
1	Background:	0	Date:	10.11.98	Time:	16:23:00
2	Comment:					
3	RLU/s	A1	A2	A3	A4	
4	0s	133	123	233	145	
5	13s	98	102	111	157	

Figure 4-5: *Presentation of the measured results in an Excel spreadsheet*

Printout

Click the Print button to print the measured data in a tabulated form. If you have entered a comment it will be printed as well.

To set the print parameters, select [**Print Setup...**] on the [**File**] menu.

To view and edit the layout before printing, select [**Print Preview**] on the [**File**] menu.

Copy

Copy measured data to the clipboard. Select the respective data with the cursor and click on this tool button. You may then paste the copied data into another Windows program.

4.5 Special Functions when Using Injectors

Initialization Prior to the first measurement (after starting the programs), you must initialize the injector unit by clicking the Initialize button. Otherwise, no measurement can be started. Then you are prompted to place a plastic tub into the microplate loading compartment (instead of a microplate) to collect any spilled liquid. As soon as you have placed the plastic tub, click <**OK**>. The end of the initialization process is again indicated in a window, while the pumps perform several lifting motions.
Take the plastic tub out and insert the microplate to be measured; then click <**OK**> in the message window. Start the measurement by clicking the green signal light.

Priming Washing and priming of the injector tubings is possible only when the injector unit has been initialized. Make sure you have placed an empty plastic tub in the microplate loading compartment to collect spilled liquid!
Click on the Prime button to open the **Parameters for Priming Injectors** dialog box.
Select the injector (**Inj. 1** or **Inj 2**).
For each injector, enter the volume/strokes (**ml**) and the number of strokes.
The programmed priming process starts as soon as you click <**OK**>. When the priming process is finished you can start the measurement using the selected measurement protocol.

Stopping the Priming Process
You may stop a started priming process any time by clicking the Stop Priming button.

4.6 Software Functions at One Glance

The **Simplicity** software is a Windows 95 application and is therefore operated according to the Windows conventions.
To simplify operation, you can use tool buttons (icons) in the measurement menu; in this case you do not need to open a pulldown menu.
The tool buttons and their functions:

Button	*Function*	*Pulldown-menu -> Option*
	Save measured data	[**File**] -> [**Save (as**)]
	Clear sample matrix for new measurement	[**File**] -> [**New**]
	Copy measured data	[**Edit**] -> [**Copy**]
	Show measured data in Excel	[**Edit**] ->[**Send to Excel**]
	Enter comment on measure-ment	[**Edit**] -> [**Measurement Comments**]
	Button for selection of wells	[**Measure**] -> [**Select wells to Measure**]
	Print	[**File**] -> [**Print**]
	Start measurement	[**Measure**] -> [**Start Measurement**]
	Stop measurement	[**Measure**] -> [**Stop Measurement**]
	Move microplate to home posi-tion	[**Measure**] -> [**Move home**]
	Run background measurement	[**Measure**] -> [**Get Background**]

	Tabulated presentation of results	[**Window**] -> [**Show Table**]
	Graphical presentation of results	[**Window**] -> [**Show Kinetics Graph**]
	Graphical presentation of individual wells	[**Window**] -> [**Graph wells**]
	Zoom function	[**Window**] -> [**Zoom**]
	Windows arranged horizontally	[**Window**] -> [**Tile Horizontal**]
	Windows arranged vertically	[**Window**] -> [**Tile Vertical**]

	Initializing the injectors
	Washing/Priming the injector tubings
	Stopping the washing / priming process

Options without buttons:

[**Options**] -> [**Autosave**]
If this item is active (indicated by a ✔), the measured data are saved automatically upon completion of the measurement. The measured data are saved according to the measurement method used to consecutively numbered files and cleared from the screen. Thus, you can start another measurement using either the selected sample area or defining a new area immediately.

[**File**] -> [**Exit**]
Exit the measurement menu and return to the **Protocol Manager**.

File names of autosave files:

Raw Data	RD1.TXT, RD2.TXT ... RDQMxxx.TXT
Slow Kinetics	SK1.TXT, SK2.TXT ... SKxxx.TXT
Fast Kinetics	FK1.TXT, FK2.TXT ... FKxxx.TXT

5. Raw Data

5.1 Overview

The function **Raw Data** allows you to run measurements with minimum preparations. The measurement starts as soon as you have entered the measurement parameters and defined the wells to be measured. Then the measured data are transferred to the PC every 0.2 seconds and the averaged results are displayed on the screen in a sample matrix. The measured results can then be imported into EXCEL for further processing.

5.2 Measurement Protocol

Creating a new measurement protocol

In the **Protocol Manager**, select the protocol type [**Raw Data**] and click <**Create...**>.

Editing an existing measurement protocol

In the **Protocol Manager**, select an existing measurement protocol and click <**Edit...**>.

The [**Raw Data PC Parameters**] dialog box appears, with the file name of the measurement protocol being displayed on the title bar. This dialog box comprises several tabs for input of the measurement parameters (Figure 5-1).

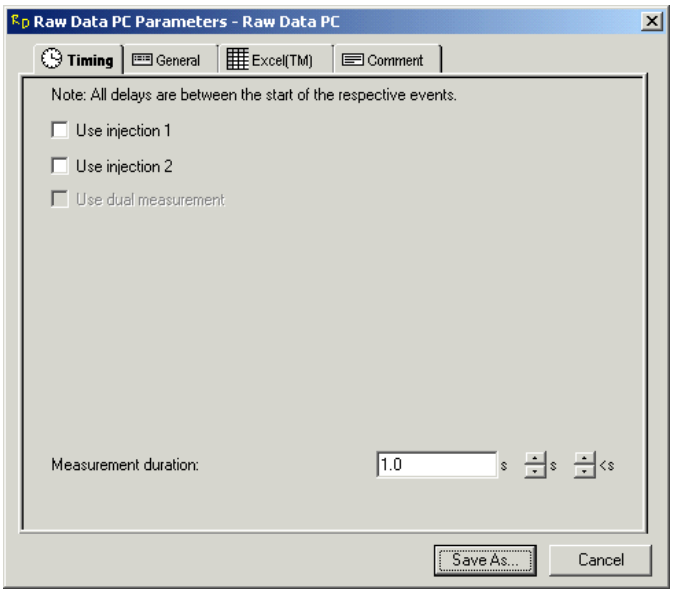


Figure 5-1: *[**Timing**] tab in the [**Raw Data PC Parameters**] dialog box*

[Timing] tab (without injectors)

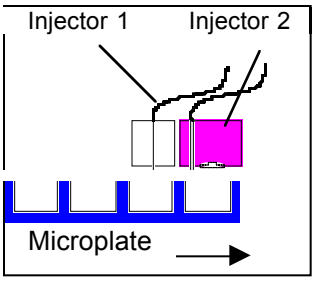
[**Measurement duration**]: Enter the measurement time per well. The default value is 1 second.

[Timing] tab (with injectors)

Except for the measurement time, you enter the parameters for the individual injections here:

When using one injector:

Select injector (**inj. 1** or **inj. 2**) by clicking on the respective box. You can then enter the injector parameters.



Please note: Injector 1 injects into the well ***before*** the measurement position, injector 2 ***in*** the measurement position.

[**Volume**]: Enter the injection volume in µl (via keyboard or by clicking on the arrow buttons).

[**Injection 1 to measurement delay**]: Enter the delay time between the injection of injector 1 and the start of the measurement in seconds. The hardware-dependent minimum value of 2.05 seconds is defaulted and can be increased.

Or:

[**Injection 2 to measurement delay**]: Enter the delay time between the injection of injector 2 and the start of the measurement in seconds. The minimum value of 0 seconds is defaulted and can be increased.

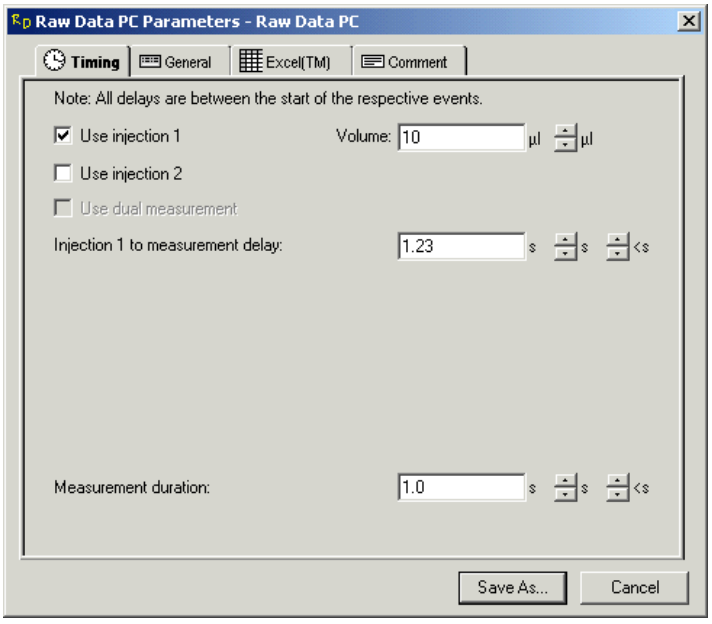
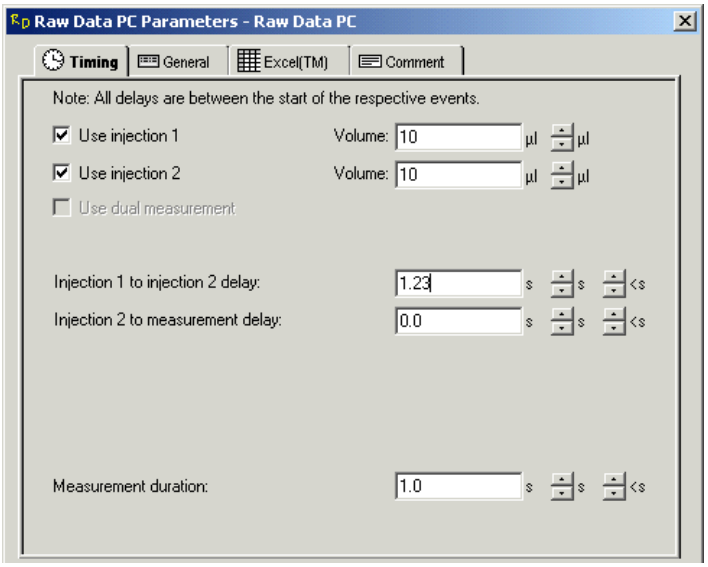


Figure 5-2: *[**Timing**] tab. Selection of injector 1*

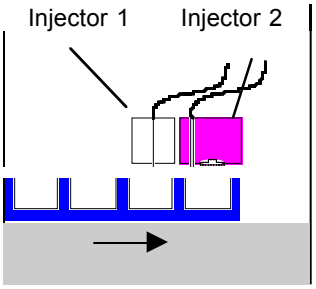
When using *two* injectors:

Select both injectors. The following dialog box appears:

Figure 5-3: *[Timing] tab. Selection of injector 1 and 2*



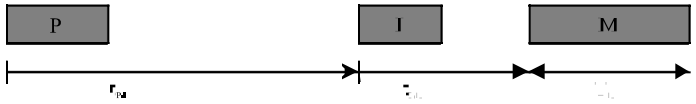
[**Volume**]: Enter the injection volumes in µl (via keyboard or by clicking on the arrow buttons).



[**Injection 1 to injection 2 delay**]: Delay time between the injections of injector 1 and 2. The hardware-dependent minimum value of 2.05 seconds is defaulted and can be increased (t_{P-I}).

[**Injection 2 to measurement delay**]: Enter the delay time between the injection of injector 2 and the start of the measurement in seconds. 0 second is defaulted and can be increased (t_{M-I}).

Example: Programming a measurement with 2 injections:



P = pre-injection
I = injection
M = measurement
t_{P-} = delay between pre-injection and 2nd injection
t_{M-I} = delay between 2nd injection and start of measurement
T_M = duration of measurement

Dual Measurement

The Simplicity 1.0 software includes four pre-programmed dual luciferase reagent (DLR) protocols. Dual measurement protocols cannot be created using the Simplicity 1.0 software, The "Use dual measurement" option is grayed-out in the screen seen in Figure 5.3.

[General] tab

Select the type of microplate you are using (**96** wells).

Select [**Take background at start of plate measurement**] if you want to run a background measurement prior to the sample measurement.

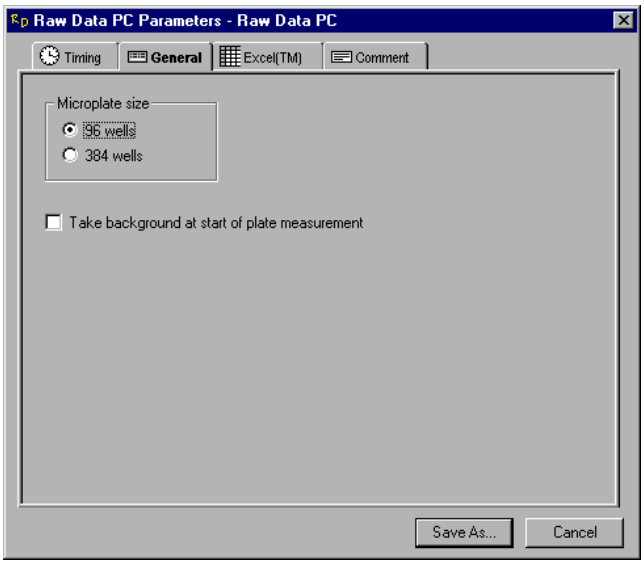
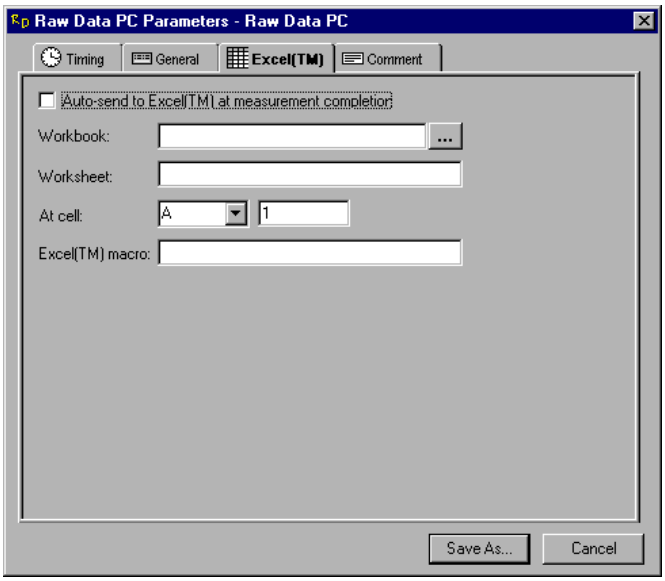


Figure 5-4: *[**General**] tab in the [**Raw Data PC Parameters**] dialog box*

[EXCEL (TM)] tab

Create an EXCEL macro for control of a measurement procedure by making the necessary entries here. Prerequisite is that EXCEL 7.0 is installed on your PC and running (Figure 5-5).

Figure 5-5: [EXCEL (TM)] tab



[**Auto-send to...**] Upon completion of the measurement, the Excel macro is automatically sent to the Excel program and executed there.

[**Workbook**] Selection of workbook. Click on <**...**> to display the browser. The subdirectory **Simplicity\workbook** is defaulted.

[**At cell**] The measured data are read starting at this cell of the Excel spreadsheets.

[**Excel(TM) macro**] Entry of an Excel macro.

[Comment] tab

The comment entered here appears, after the protocol has been saved, in the **Protocol Manager** next to the measurement protocol list when the respective measurement protocol is selected.

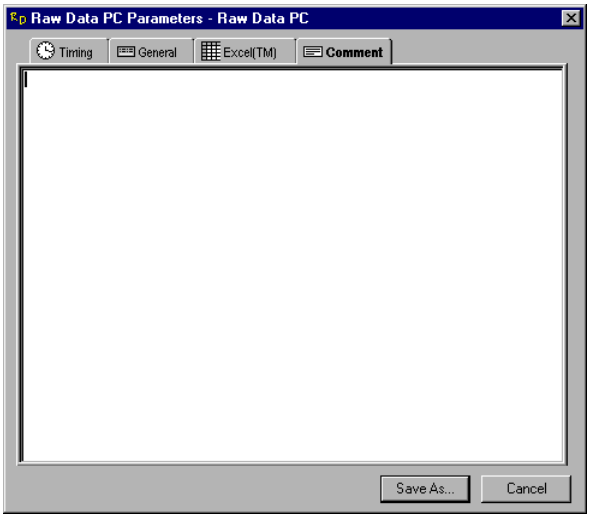


Figure 5-6: *Input field for a comment on the measurement protocol*

The following buttons appear on all tabs of the entire measurement protocol:

<Save As...> is selected to define a new file name. This is necessary in order to create a new measurement protocol, or to save an existing measurement protocol under a new name. Clicking on this button opens the [**Save As...**] dialog box. Enter a file name which differs by at least one character from the file name of the selected protocol type. As soon as the protocol has been saved, the program returns to the **Protocol Manager** and shows the name of the new measurement protocols in the field [**Runable protocols**].

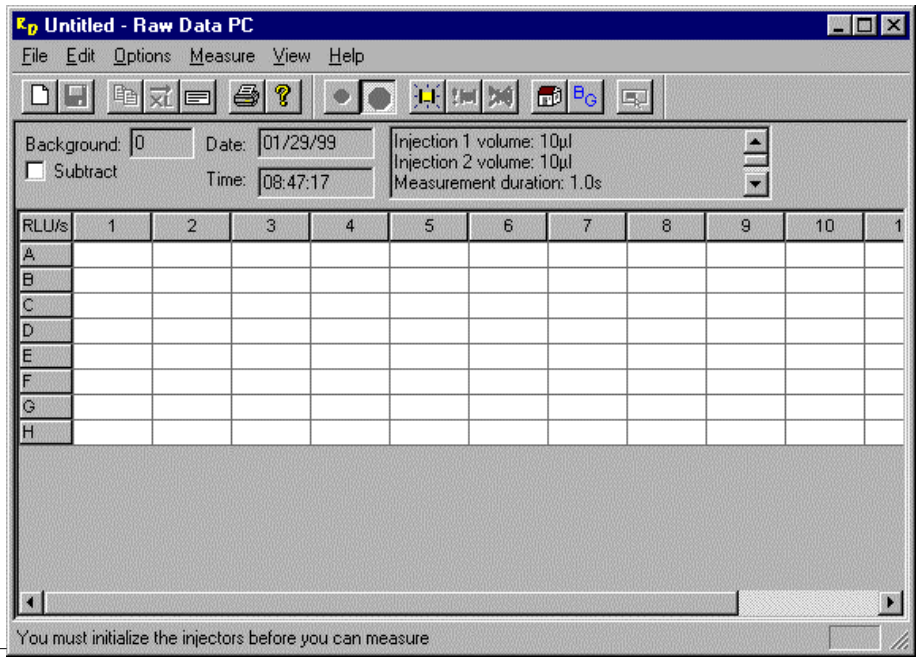
<Save> Saves the parameters in the existing measurement protocol file. After you have saved the protocol, the program returns to the **Protocol Manager**.

<Cancel> The changes are disregarded and the old values are still valid. The program returns to the **Protocol Manager**.

5.3 Measurement

As soon as you have selected measurement protocol type **Raw Data PC** and you have clicked **<Run>**, the program changes to the [**Raw Data PC**] measurement menu. Communication with the Microplate Luminometer is established (if this has not been done during program start). "**Initializing instrument**" appears on the status bar and the yellow LED lights up.

Figure 5-7: *Measurement menu* ***[Raw Data PC]***



Please note: Before running a measurement, if desired, select the autosave mode. Select this function in the [**Options**] menu.

Procedure (without injections)

❏ Insert microplate correctly into the luminometer and close cover.

❏ Run background measurement, if necessary ( ).

❏ Select the wells to be measured ( ).

❏ Start measurement ( ).

Procedure with injections

- ❑ Select the wells to be measured.
- ❑ Place plastic tub in plate loading compartment.
- ❑ Initialize injectors ().
- ❑ Prime injector tubing, if necessary (). Insert microplate.
- ❑ Insert microplate correctly in luminometer and close door.
- ❑ If necessary, run background measurement ().
- ❑ Select the wells to be measured ().
- ❑ Start measurement ().

Measurement start

Measurement of the microplate or the selected area starts after pressing the Start button (green signal light), when the instrument door is closed.

The Start button is dimmed (gray) when a measurement is running, or no communication has been established with the luminometer, or the data of the last measurement have not yet been saved. In all these cases, no measurement can be started.

If the instrument door is not closed, a message is displayed indicating the current measurement mode:

Close the instrument door and click <**Repeat**> or <**OK**>. Then the measurement will be started.

Measurement procedure

Any programmed injections will be performed and the selected wells are measured one after the other. The results of the individual samples are entered into the respective fields.

Measurement end The measurement is finished as soon as all selected wells have been measured. In the autosave mode, the measured results are automatically saved and the measured values cleared from the screen. If the autosave mode is not active the measured values remain displayed and can be saved.

Measurement stop Push the Stop button (red signal light) to stop a measurement. The results measured so far can be stored.

6. Slow Kinetics

6.1 Overview

In the measurement mode **Slow Kinetics** the trend of the light emission of samples is measured. To this end, the number of data points must be defined. The measurement starts after input of the measurement parameters and definition of the wells to be measured. Then all samples for data point 1 are measured in succession and the averaged results displayed in a table. Then the measurement is carried out for data point 2 etc.
The data can be presented as a graph or in a table or imported into an EXCEL spreadsheet for further processing.

6.2 Measurement Protocol

Creating a new measurement protocol

In the **Protocol Manager**, select the protocol type [**Slow Kinetics**] and click <**Create...**>.

Editing an existing measurement protocol

In the **Protocol Manager**, select an existing measurement protocol and click <**Edit...**>.

The [**Slow Kinetics Parameters**] dialog box with several tabs is displayed and you can enter the measurement parameters (Figure 6-1).

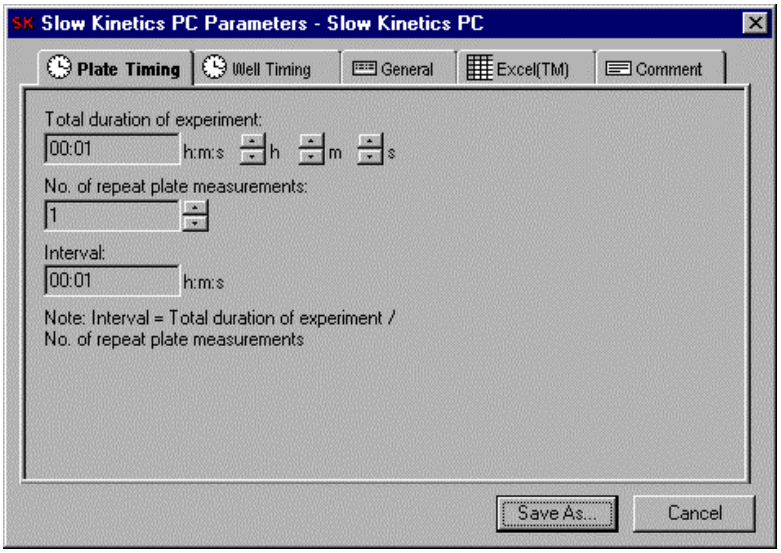


Figure 6-1: *[**Plate Timing**] tab in the [**Slow Kinetics**] dialog box*

[Plate Timing] tab

The following mutually dependent parameters must be taken into account for the kinetics measurement:

- the number of data points
- measurement time per well = 1 sec
- the number of samples to be measured
- the number of injections per well
- the delay time between injection(s) and measurement time
- the expected duration of the light emission of the samples

- the time required for the movement of the microplate transport unit. This is in turn dependent upon the position of the samples to be measured. If the samples are located all over the microplate, more time is needed than for an arrangement in one row or in one corner of the microplate (at least 1 sec).

Please note: for each data point (interval), all selected samples are measured in succession.

Calculation of actual times:

Total duration of experiment:	Duration of the interval x number of data points.
Duration of interval:	(Delay times + measurement time + feed time) x number of samples.

[**Total duration of experiment**]
Total duration of measurement (including movement of the microplate transport unit)

[**No. of repeat plate measurement**]
Number of data points.

[**Interval**]
The program shows the interval period calculated from the two previous parameters. During this time, the defined samples should be measured once. The minimum duration should be ***number of samples x 2 sec***.

Parameter input

The interval is defined by changing the parameters [**Total duration...**] and [**No. of repeat...**]. The program calculates the inputs in the dialog box according to the formula

[**Interval**] = [**Total duration...**]/[**No. of repeat...**]

This demonstrates that the total duration must be increased when the interval is to be extended while the number of data points stays the same.

Please note: Use intervals that are long enough to allow for the microplate transport.

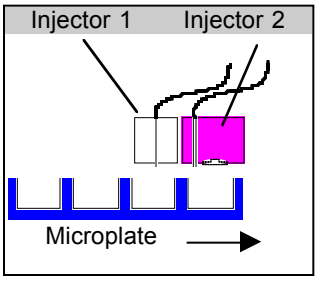
The program automatically corrects for intervals that are too short:

If you have defined an interval that is too short, the program calculates the required intervals using the new time actually required, and displays it on the screen after measurement of the 1st data point. The measurement can then be continued with the new parameters or aborted. For example, if you start a measurement with 2 samples using the parameters from Figure 6-2, the time data is changed upon completion of the 1st interval: depending on the position of the selected wells the data points are e.g. 0 s, 6 s, 12 s (instead of 0 s, 1 s, 2 s).

[Well Timing] tab (with injectors)

Except for the measurement time, enter the parameters for the individual injections here:

When using *one* injector:



Select the injector (**inj. 1** or **inj. 2**) by clicking on the respective box. Then enter the injector parameters.

Please note: Injector 1 injects into the well ***before*** the measurement position, injector 2 ***in*** the measurement position.

[**Volume**]: Enter the injection volume in µl (via keyboard or by clicking on the arrow buttons).

[**Injection 1 to measurement delay**]: Enter the delay time between the injection of injector 1 and the start of the measurement in seconds. The hardware-dependent minimum value of 2.05 seconds is defaulted and can be increased.

Or:

[**Injection 2 to measurement delay**]: Enter the delay time between the injection of injector 2 and the start of the measurement in seconds. The minimum value of 0 seconds is defaulted and can be increased.

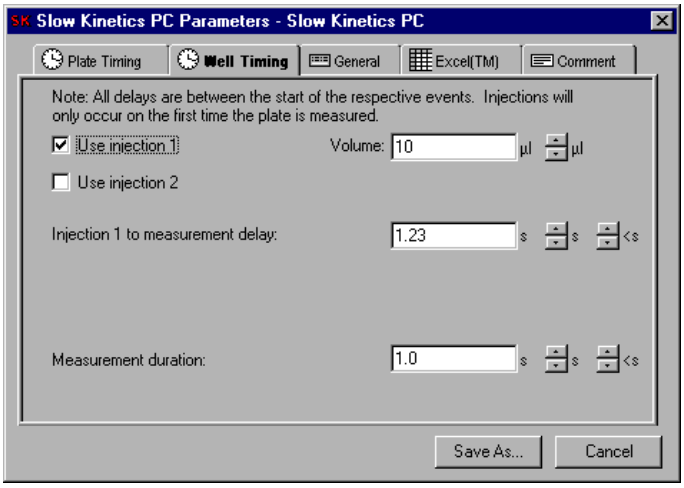


Figure 6-3: *[**Well Timing**] tab. Selection of injector 1*

When using *two* injector:

Select both injectors. The following dialog box appears:

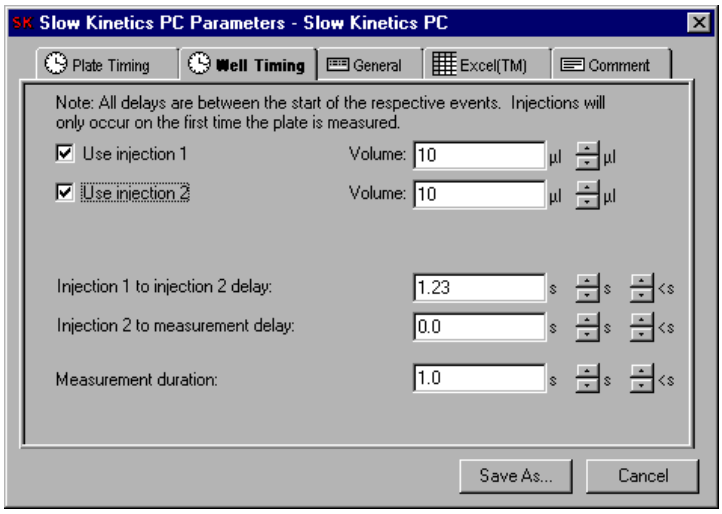
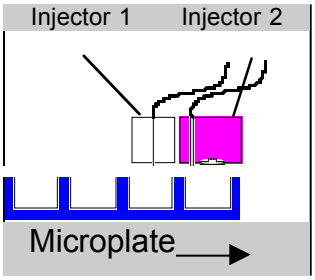


Figure 6-4: *[**Well Timing**] tab. Selection of injector 1 and 2*

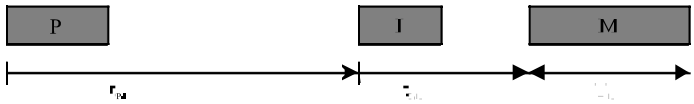


[**Volume**]: Enter the injection volumes in µl (via keyboard or by clicking on the arrow buttons).

[**Injection 1 to injection 2 delay**]: Delay time between the injections of injector 1 and 2. The hardware-dependent minimum value of 2.05 seconds is defaulted and can be increased (t_{P-I}).

[**Injection 2 to measurement delay**]: Enter the delay time between the injection of injector 2 and the start of the measurement in seconds. 0 second is defaulted and can be increased (t_{M-I}).

Example: Programming a measurement with 2 injections:



P = pre-injection
I = injection
M = measurement
t_{P-I} = delay between pre-injection and 2nd injection
t_{M-I} = delay between 2nd injection and start of measurement
T_M = duration of measurement

Dual Measurement

If this item is checked, each selected sample is measured twice and both measured values are displayed in the respective field. This function can be used with or without injectors. Parameters must be entered accordingly:

No injector:	Delay time: **1st measurement -> 2nd measurement**
Injector 1:	Delay times: **Inj. 1 -> 1st measurement -> 2nd measurement**
Injector 2:	Delay times: **1st measurement -> Inj. 2 -> 2nd measurement**
Injector 1&2:	Delay times: **Inj. 1 -> 1st measurement -> Inj. 2 -> 2nd measurement**

[General] tab

Select the type of microplate you are using (**96** wells).

Select [**Take background at start of plate measurement**] if you want to run a background measurement prior to the sample measurement.

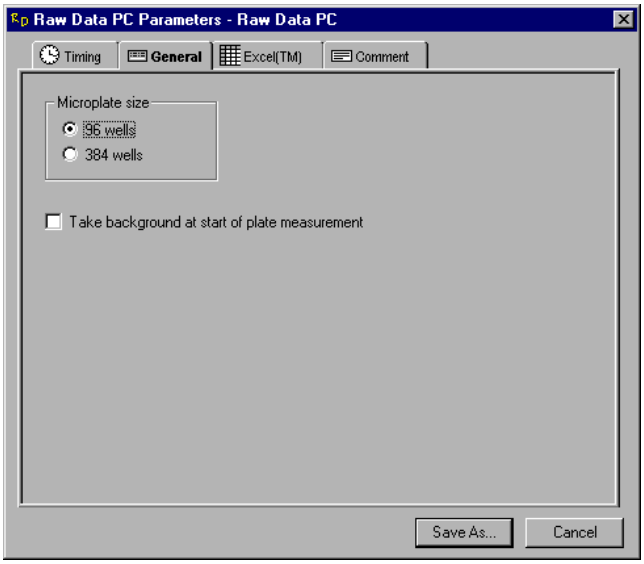


Figure 6-5: *[General] tab in the* ***[Raw Data PC Parameters]*** *dialog box*

[EXCEL (TM)] tab

Create an EXCEL macro to control a measurement, by making the necessary entries here. Prerequisite is that EXCEL 7.0 is installed on your PC and running (Figure 6-6).

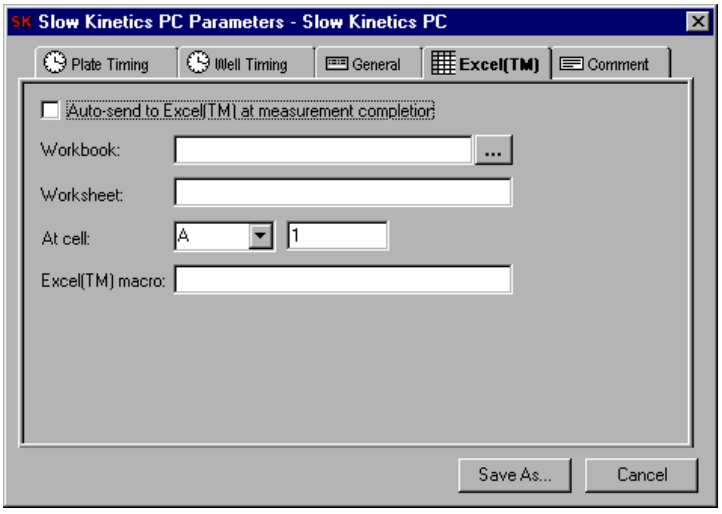


Figure 6-6: *[EXCEL (TM)] tab*

[**Auto-send to...**] Upon completion of the measurement, the Excel macro is automatically sent to the Excel program and executed there.

[**Workbook**] Selection of workbook. Click on <**...**> to display the browser. The subdirectory **Simplicity\workbook** is defaulted.

[**At cell**] The measured data are read, starting at this cell of the Excel spreadsheets

[**Excel(TM) macro**] Entry of an Excel macro.

[Comment] tab

The comment entered here appears, after the protocol has been saved, in the **Protocol Manager** next to the measurement protocol list when the respective measurement protocol is selected.

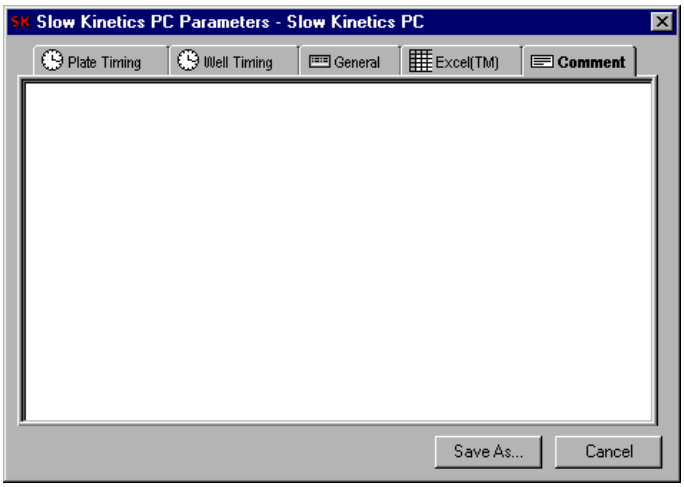


Figure 6-7: *Input field for a comment on the measurement protocol*

The following buttons appear on all tabs of the entire measurement protocol:

<**Save As...**> is selected to define a new file name. This is necessary in order to create a new measurement protocol, or to save an existing measurement protocol under a new name. Clicking on this button opens the [**Save As...**] dialog box. Enter a file name which differs by at least one character from the file name of the selected protocol type. As soon as the protocol has been saved, the program returns to the **Protocol Manager** and shows the name of the new measurement protocols in the field [**Runable protocols**].

<Save> Saves the parameters in the existing measurement protocol file. After you have saved the protocol, the program returns to the **Protocol Manager**.

<Cancel> The changes are disregarded and the old values are still valid. The program returns to the **Protocol Manager**.

6.3 Measurement

As soon as you have selected the measurement protocol type **Slow Kinetics** and you have clicked **<Run>**, the program changes to the measurement menu [**Slow Kinetics**]. Communication with the Microplate Luminometer is established. "**Initializing instrument**" appears on the status bar and the yellow LED lights up.

In the measurement menu [**Slow Kinetics**] the sample matrix is displayed first, and below that a table for the measured values. The table includes, row by row, the predefined data points for ***all*** samples on the microplate (see Figure 6-8). A measurement of the entire plate can also be started immediately by pressing the Start button.

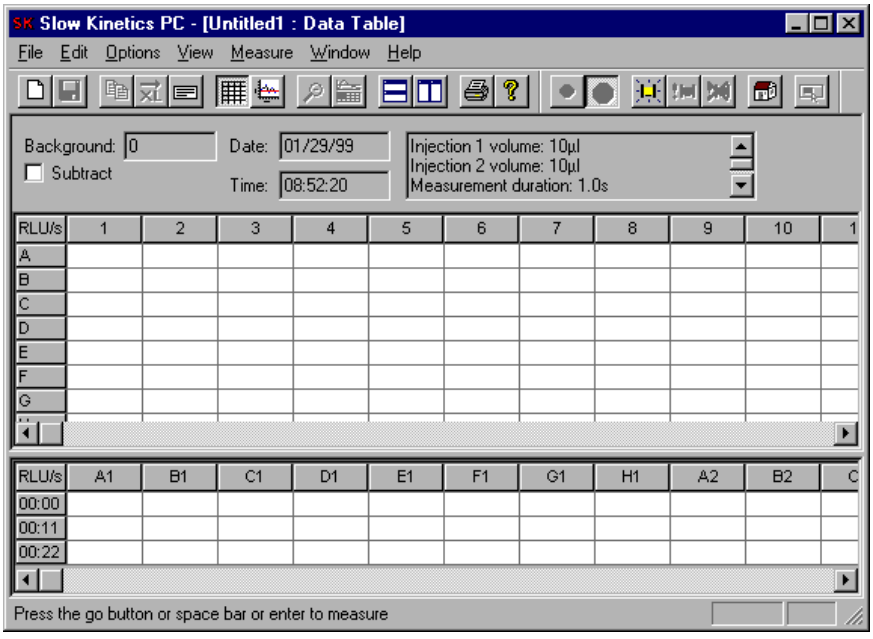
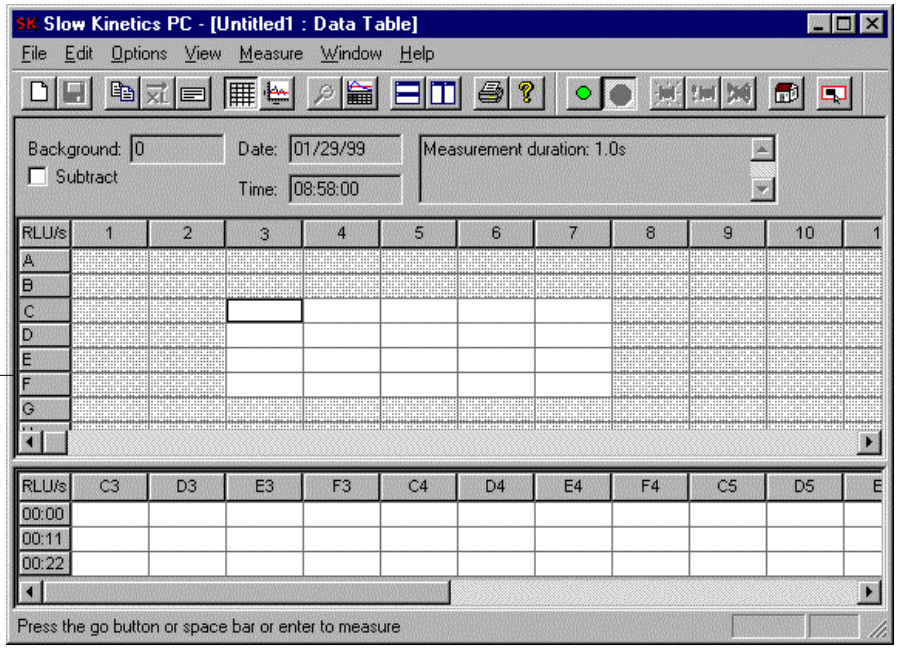


Figure 6-8: *Measurement menu [**Slow Kinetics**] with sample matrix and table of measured values*

Selecting the wells to be measured

❑ Mark the well group you want with the mouse and press the  button to confirm your selection. The selected wells are presented white and the measurement value table is displayed according to the selected wells with data points (see Figure 6-9).

Figure 6-9: *Measurement menu [**Slow Kinetics**] with selected well groups*



Measurement value table with predefined intervals. They are changed by the program upon completion of the first interval if they are too short.

Please note: Before running a measurement, if desired, select the autosave mode. Select this function on the [**Options**] menu.

Procedure without injectors

- ❑ Insert microplate correctly into the luminometer and close cover.
- ❑ Run background measurement, if necessary ( ).
- ❑ Select the wells to be measured ( ).
- ❑ Start measurement ( ).
- ❑ Data point 1: all (selected) samples are measured in succession and the measured values are displayed on the screen. If you have selected too short intervals, the system corrects the intervals using the actually required time.
- ❑ Then the measurements at data point 2 are performed and the results displayed. All following measurements are performed in the same manner.
- ❑ The measurement is finished when all predefined measurements have been carried out (Figure 6-10).

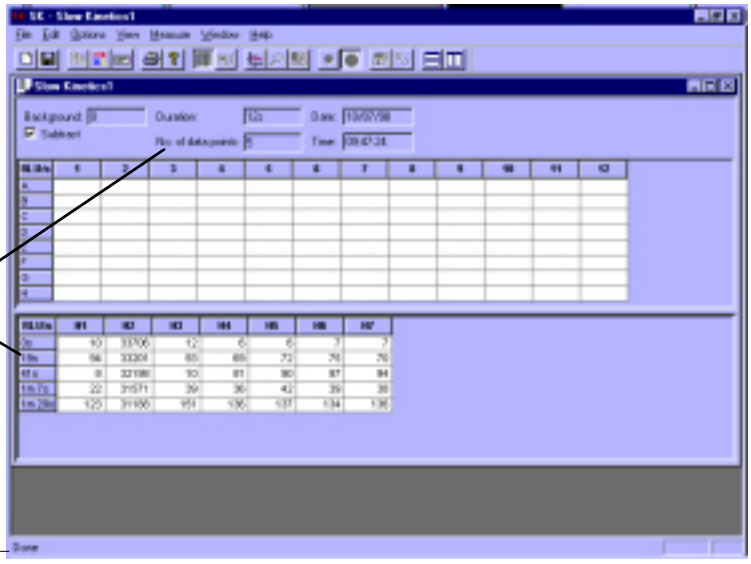


Figure 6-10: *Measurement menu [**Slow Kinetics**] with tabulated result display*

Procedure with injections

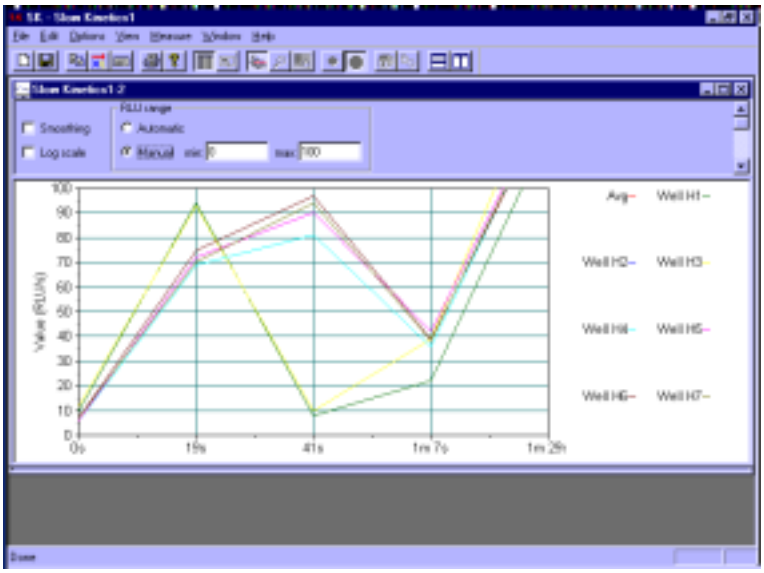
- ❑ Select the wells to be measured.
- ❑ Place plastic tub in plate loading compartment.
- ❑ Initialize injectors ().
- ❑ Prime tubings, if necessary (). Insert microplate.
- ❑ Insert microplate correctly in luminometer and close door.
- ❑ If necessary, run background measurement ().
- ❑ Select the wells to be measured ().
- ❑ Start measurement ().
- ❑ Data point 1: The pre-defined injections and the measurements of the selected samples are carried out in succession and the measured values are displayed on the screen. If you have selected too short intervals, the system corrects the intervals using the actually required time.
- ❑ Then the measurements at data point 2 are performed and the results displayed. All following measurements are performed in the same manner.
- ❑ The measurement is finished when all predefined measurements have been carried out (Figure 6-10).

6.4 Result Display

Graphical and tabulated display of the measured results

As needed, you can call the window for graphical display of the measured data before, during or after completion of the measurement by clicking  . The graph display is on top of the table window (Figure 6-11).

Figure 6-11: *Measurement menu* ***[Slow Kinetics]*** *with graphical result display*



- To display the curve trend of one or several selected samples, select the desired wells in the sample matrix with the cursor and click  .
- To view the graph and table window on the screen at the same time, click  or  - depending on the layout you prefer (Figure 6-12).

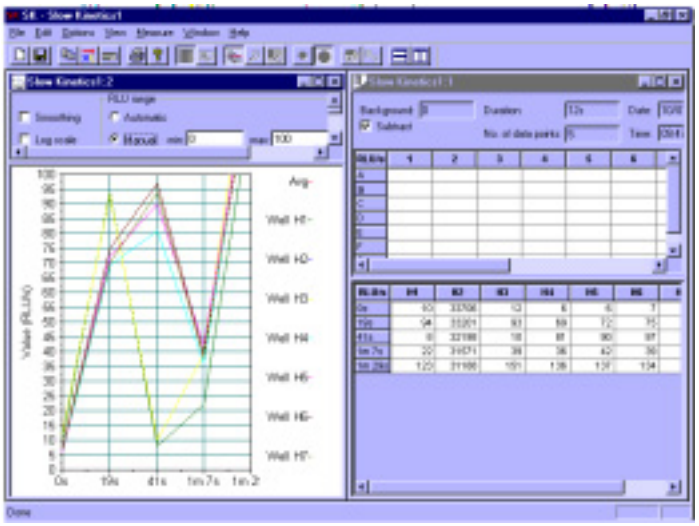


Figure 6-12: *Measurement menu [**Slow Kinetics**] with tabulated and graphical result display*

- To open or close one display mode, click the button  or  . The remaining window will fill half the screen.
- To maximize the window, click  or  .

Further options for opening/closing/arranging/maximizing/minimizing the size of graph and table windows:

Buttons in the upper right corner of the dialog box:

 Maximizing the windows to full size.

 Minimizing the windows to half the screen size.

Buttons in the upper left corner of the dialog box:

 Opens a pulldown menu including the item [**Close**] to close the table window.

 Opens a pulldown menu including the item [**Close**] to close the graph window.

Maximizing/Minimizing graphs

In the graph window, grab the horizontal bar dividing the graphics and data section with the mouse and pull it down. Thus, additional items as well as the listing of the measured value with calculations become visible (average, peak, etc.). You may also increase the graphics section by moving the horizontal bar up so far that the entire data section is hidden.

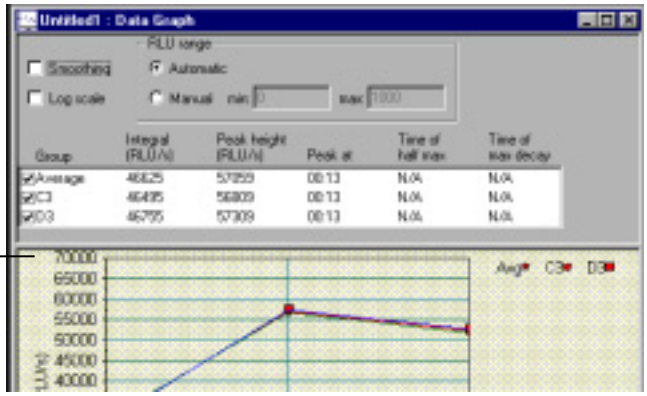


Figure 6-13: *Graph window [**Slow Kinetics**] (partial view)*

Here you may pull the dividing bar up or down with the mouse

The graph window

The curve trend of the measured values is presented in the graph window (default setting: automatic scaling). The well positions and associated colors are listed in the graphics legend next to the diagram.

Options in the graph window

[**Smoothing**]	Smoothing the measured values
[**Log scale**]	Logarithmic scaling of the measured values
[**RLU range**]	Selection of scaling:
[**Automatic**]	Default setting is automatic scaling of the measured RLU values, comprising the top and bottom value of each coordinate.
[**Manual**]	Manual setting option for the display area. Upon selection of this item you may enter the start ([**min**]) and end ([**max**]) of the RLU ranges. When you click [**Manual**] once more, the entered range can be applied to the graphics.

7. Fast Kinetics

7.1 Overview

In the measurement mode **Fast Kinetics** the trend of the brief light emission of samples is measured. The individual samples are measured in succession and the measured results are displayed in a table. The data can be presented as a graph or in a table or imported into an EXCEL spreadsheet for further processing.

7.2 Measurement Protocol

Creating a new measurement protocol

In the **Protocol Manager**, select the protocol type [**Fast Kinetics**] and click <**Create...**>.

Editing an existing measurement protocol

In the **Protocol Manager**, select an existing measurement protocol and click <**Edit...**>.

The [**Fast Kinetics Parameters**] dialog box with several tabs is displayed and you can enter the measurement parameters (Figure 7-1).

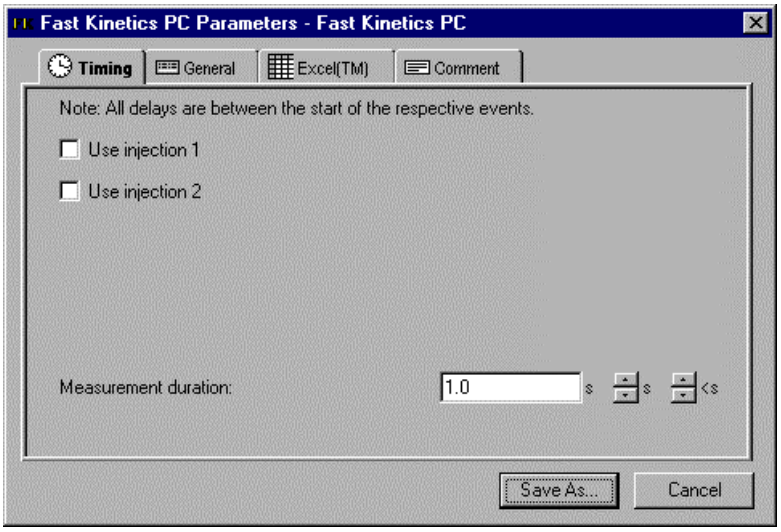


Figure 7-1: *[**Timing**] tab in the [**Fast Kinetics PC Parameters**] dialog box*

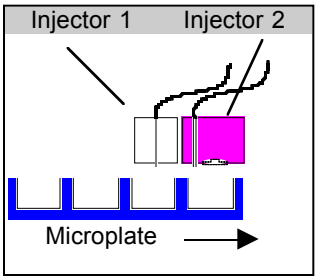
[Well Timing] tab (without injectors)

[**Measurement duration**]: Enter the measuring time per well. The default value is 1 second.

[Well Timing] tab (with injectors)

Except for the measurement time, enter the parameters for the individual injections here:

When using *one* injector:



Select the injector (**inj. 1** or **inj. 2**) by clicking on the respective box. Then enter the injector parameters.

Please note: Injector 1 injects into the well ***before*** the measurement position, injector 2 ***in*** the measurement position.

[**Volume**]: Enter the injection volume in µl (via keyboard or by clicking on the arrow buttons).

[**Injection 1 to measurement delay**]: Enter the delay time between the injection of injector 1 and the start of the measurement in seconds. The hardware-dependent minimum value of 2.05 seconds is defaulted and can be increased.

Or:

[**Injection 2 to measurement delay**]: Enter the delay time between the injection of injector 2 and the start of the measurement in seconds. The minimum value of 0 seconds is defaulted and can be increased.

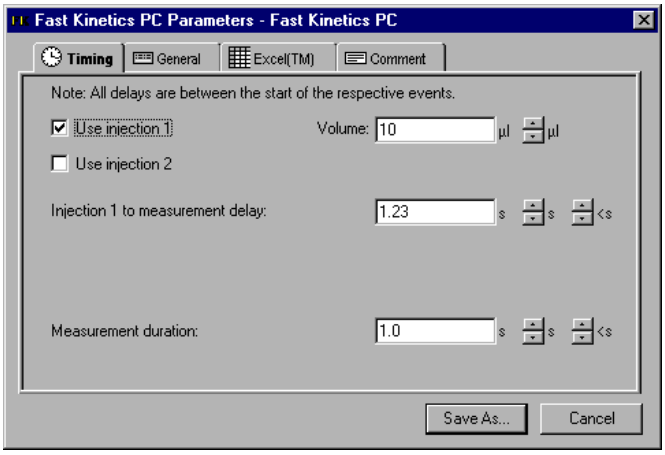
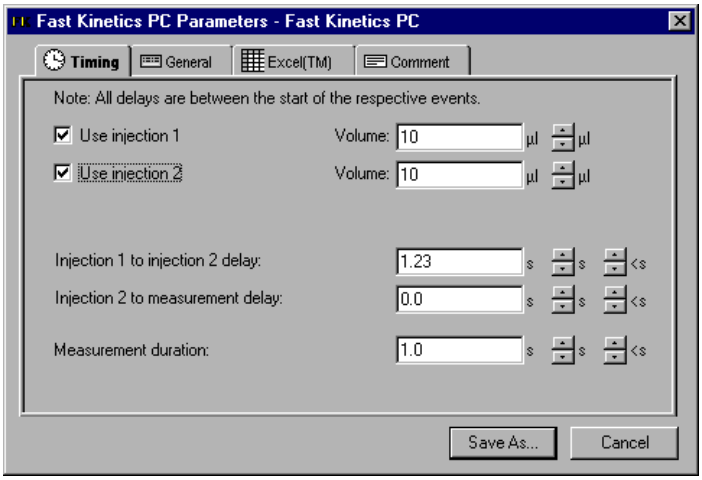


Figure 7-2: *[**Timing**] tab. Selection of injector 1*

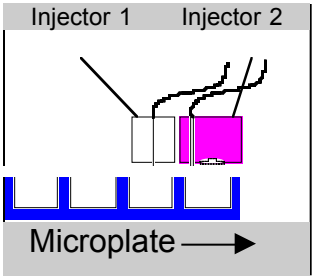
When using *two* injector:

Select both injectors. The following dialog box appears:

Figure 7-3: *[**Well Timing**] tab. Selection of injector 1 and 2*



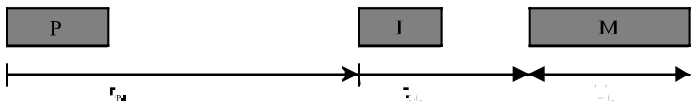
[**Volume**]: Enter the injection volumes in µl (via keyboard or by clicking on the arrow buttons).



[**Injection 1 to injection 2 delay**]: Delay time between the injections of injector 1 and 2. The hardware-dependent minimum value of 2.05 seconds is defaulted and can be increased (t_{P-I}).

[**Injection 2 to measurement delay**]: Enter the delay time between the injection of injector 2 and the start of the measurement in seconds. 0 second is defaulted and can be increased (t_{M-I}).

Example: Programming a measurement with 2 injections:



P = pre-injection
I = injection
M = measurement
t_{P-I} = delay between pre-injection and 2nd injection
t_{M-I} = delay between 2nd injection and start of measurement
T_M = duration of measurement

[General] tab

Select the type of microplate you are using (**96** wells).

Select [**Take background at start of plate measurement**] to run a background measurement prior to the sample measurement.

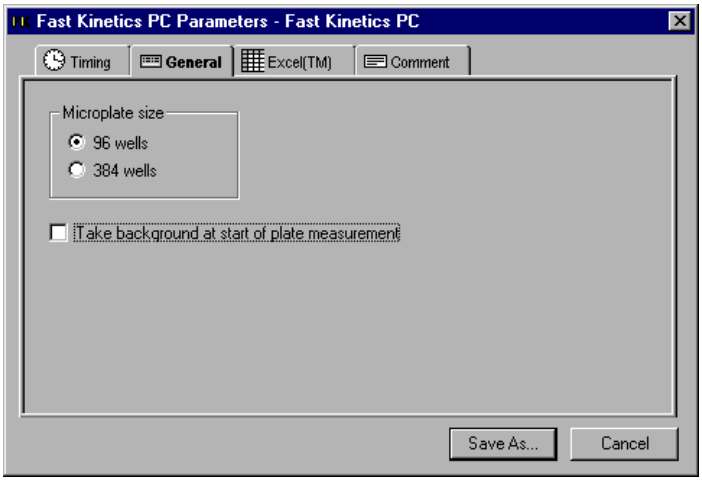


Figure 7-4: *[**General**] tab in the [**Fast Kinetics PC Parameters**] dialog box*

[EXCEL (TM)] tab

Create an EXCEL macro to control a measurement by making the necessary entries here. Prerequisite is that EXCEL 7.0 is installed on your PC and running (Figure 7-5).

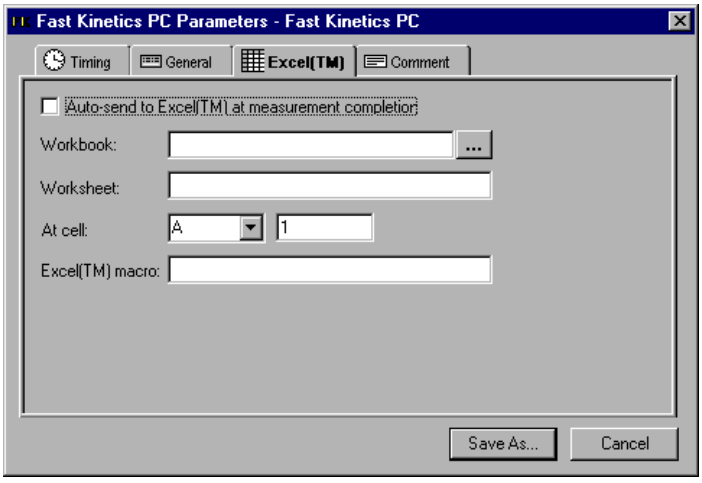


Figure 7-5: *[**EXCEL TM**] tab*

[**Auto-send to...**] Upon completion of the measurement, the Excel macro is automatically sent to the Excel program and executed there.

[**Workbook**] Selection of workbook. Click on <**...**> to display the browser. The subdirectory **Simplicity\workbook** is defaulted.

[**At cell**] The measured data are read starting at this cell of the Excel spreadsheets

[**Excel(TM) macro**] Entry of an Excel macro.

[Comment] tab

The comment entered here appears, after the protocol has been saved, in the **Protocol Manager** next to the measurement protocol list when the respective measurement protocol is selected.

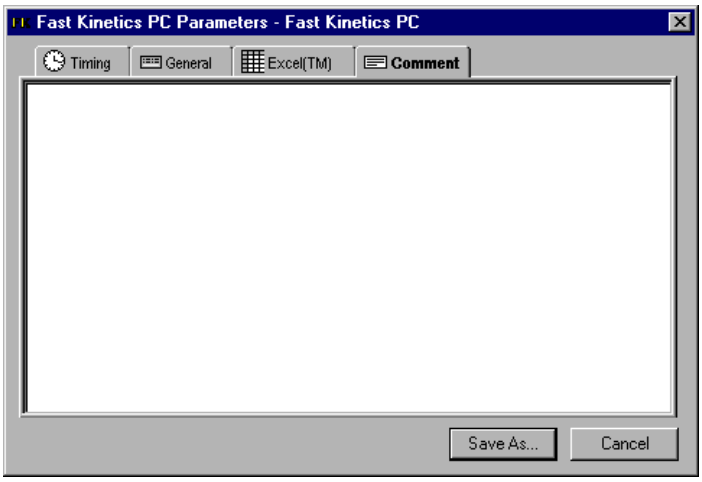


Figure 7-6: *Input box for a comment on the measurement protocol*

The following buttons appear on all tabs of the entire measurement protocol:

<Save As...> is selected to define a new file name. This is necessary in order to create a new measurement protocol, or to save an existing measurement protocol under a new name. Clicking on this button opens the [**Save As...**] dialog box. Enter a file name which differs by at least one character from the file name of the selected protocol type. As soon as the protocol has been saved, the program returns to the **Protocol Manager** and shows the name of the new measurement protocols in the field [**Runable protocols**].

<**Save**>	Saves the parameters in the existing measurement protocol file. After you have saved the protocol, the program returns to the **Protocol Manager**.
<**Cancel**>	The changes are disregarded and the old values are still valid. The program returns to the **Protocol Manager**.

7.3 Measurement

As soon as you have selected the measurement protocol type **Fast Kinetics** and you have clicked <**Run**>, the program changes to the measurement menu [**Fast Kinetics**]. Communication with the Microplate Luminometer is established, provided this has not already been done at the start of the program). "**Initializing instrument**" appears on the status bar and the yellow LED lights up.
In the measurement menu [**Fast Kinetics**] the sample matrix is displayed first and below that a table for the measured values. The table includes, row by row, the predefined data points for ***all*** samples on the microplate (see Figure 7-7). A measurement of the entire plate can also be started immediately by pressing the Start button.

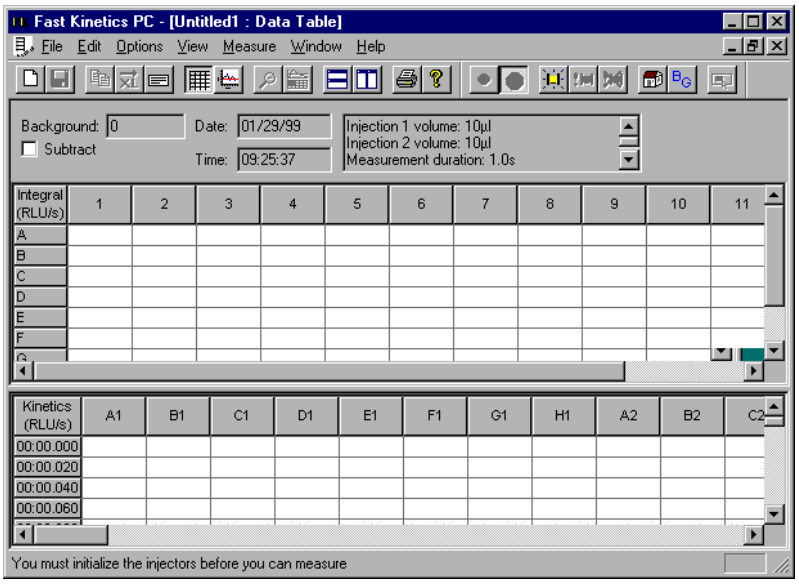


Figure 7-7: *Measurement menu [**Fast Kinetics**] wit sample matrix and table of measured values*

Selecting the wells to be measured

- ❑ Mark the well group you want with the mouse and press the button to confirm your selection.
- ❑ The selected wells are presented white and the measurement value table is displayed according to the selected wells with data points (see Figure 7-8).

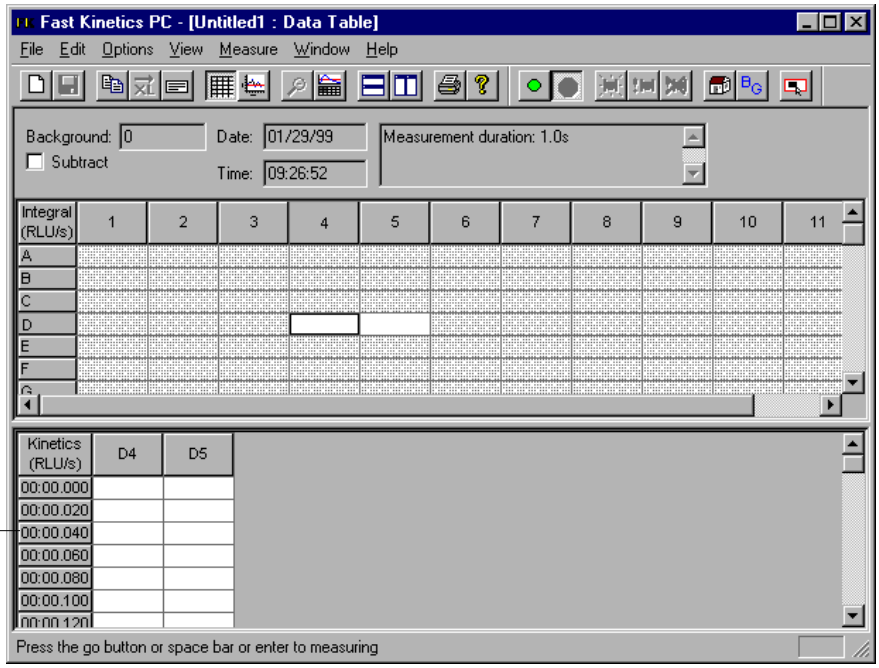


Figure 7-8: *Measurement menu [**Fast Kinetics**] with selected well groups*

Result table with data points corresponding to the pre-defined measurement times.

Please note: Before running a measurement, if desired, select the autosave mode. Select this function on the [**Options**] menu.

Procedure without injections

- ❑ Insert microplate correctly into the luminometer and close cover.
- ❑ Run background measurement, if necessary ( ).
- ❑ Select the wells to be measured ( ).
- ❑ Start measurement ( ).
- ❑ Each sample is measured according to the pre-defined measurement time, and the measured values are displayed per data point.
- ❑ The measurement is finished when all predefined measurements have been carried out (Figure 7-9).

Procedure with injections

- ❑ Select the wells to be measured.
- ❑ Place plastic tub in plate loading compartment.
- ❑ Initialize injectors ( ).
- ❑ Prime tubings, if necessary ( ). Insert microplate.
- ❑ Insert microplate correctly in luminometer and close door.
- ❑ If necessary, run background measurement ( ).
- ❑ Select the wells to be measured ( ).
- ❑ Start measurement ( ).
- ❑ The preselected injections are performed and each sample is measured in succession according to the preselected measurement time. The measured values are presented for each data point.
- ❑ The measurement is finished when all predefined measurements have been performed (Figure 7-9).

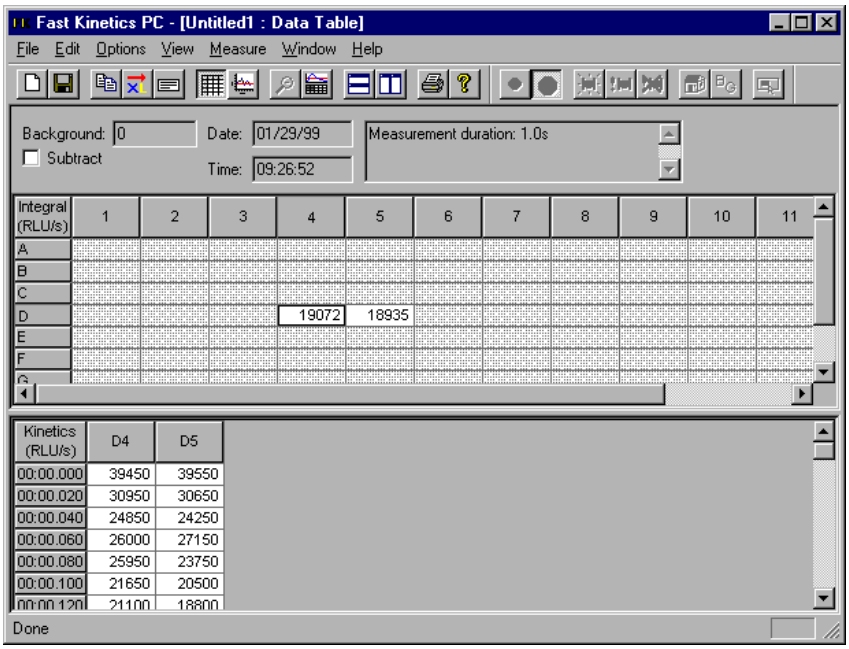


Figure 7-9: *Measurement menu **[Fast Kinetics]** with tabulated result display*

7.4 Result Display

Graphical and tabulated display of the measured results

- As needed, you can call the window for graphical display of the measured data before, during or after completion of the measurement by clicking  . The graph display is on top of the table window.

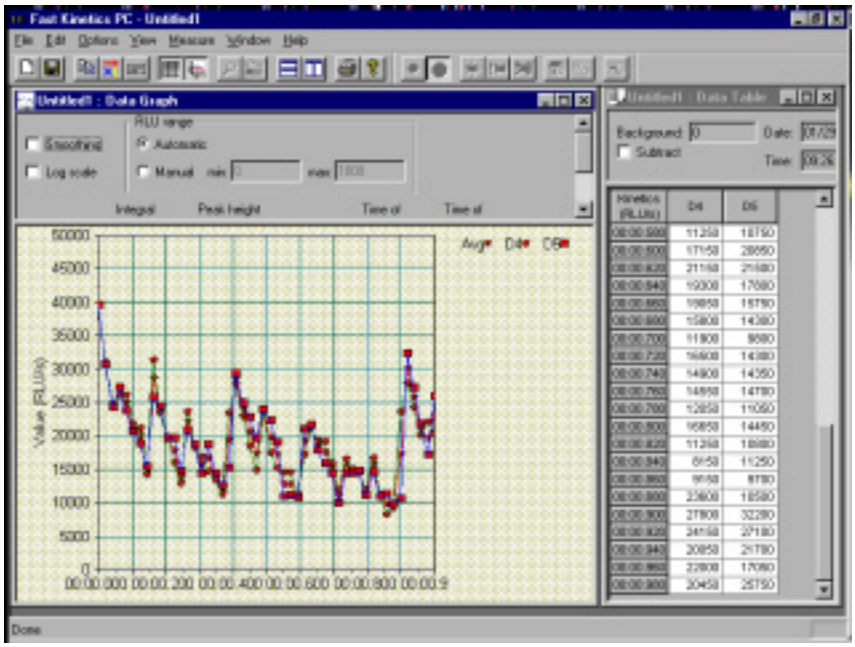


Figure 7-10: *Measurement menu [**Fast Kinetics**] with graphical and tabulated result display*

- To display the curve trend of one or several selected samples, select the wells in the sample matrix with the cursor and click  .
- To view the graph and table window on the screen at the same time, click  or  - depending on the layout you prefer (Figure 7-10).
- To open or close one display mode, click the button  or  . The remaining window will fill half the screen.
- To maximize the window, click  or  .

Further options for opening/closing/arranging/maximizing/minimizing the size of graph and table windows:

Buttons in the upper right corner of the dialog box:

Maximizing the windows to full size.

Minimizing the windows to half the screen size.

Buttons in the upper left corner of the dialog box:

Opens a pulldown menu including the item [**Close**] to close the table window.

Opens a pulldown menu including the item [**Close**] to close the graph window.

Maximizing/Minimizing graphics

In the graph window you can grab the horizontal bar dividing the graphics and data section with the mouse and pull it down. Thus, additional items as well as the listing of the measured value with the calculations become visible (average, peak, etc.). You may also increase the graphics section by moving the horizontal bar up so far that the entire data section is hidden.

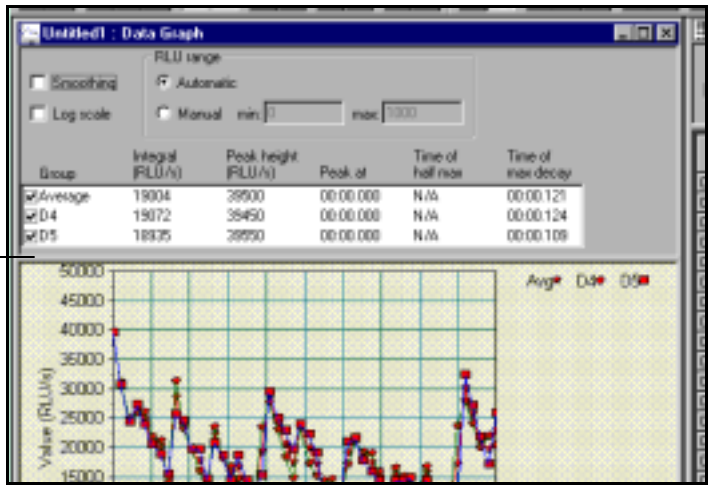


Figure 7-11: *Graph window [**Slow Kinetics**] (partial view)*

Here you may pull the dividing bar up or down with the mouse

The graph window

The curve trend of the measured values is presented in the graph window (default setting: automatic scaling). The well positions and associated colors are listed in the graphics legend next to the diagram.

Options in the graph window

[**Smoothing**]	Smoothing the measured values
[**Log scale**]	Logarithmic scaling of the measured values
[**RLU range**]	Selection of scaling:
[**Automatic**]	Default setting is automatic scaling of the measured RLU values, comprising the top and bottom value of each coordinate.
[**Manual**]	Manual setting option for the display area. Upon selection of this item you may enter the start ([**min**]) and end ([**max**]) of the RLU ranges. When you click [**Manual**] once more, the entered range can be applied to the graphics.

8. Maintenance

8.1 Cleaning the MONOLIGHT 3096

The outside of the luminometer is protected by a rugged, washable varnish. If it is dirty or dusty, clean it using a moist cloth. If necessary, use a mild cleaner (e.g. detergent, but never any scouring powder!).

Keep the microplate transport unit and frame clean.
The spring-loaded, black microplate support tray is accessible when the instrument is open and the frame is turned up. If it is dirty, clean it using a moist cloth and use cotton swabs to clean the corners.

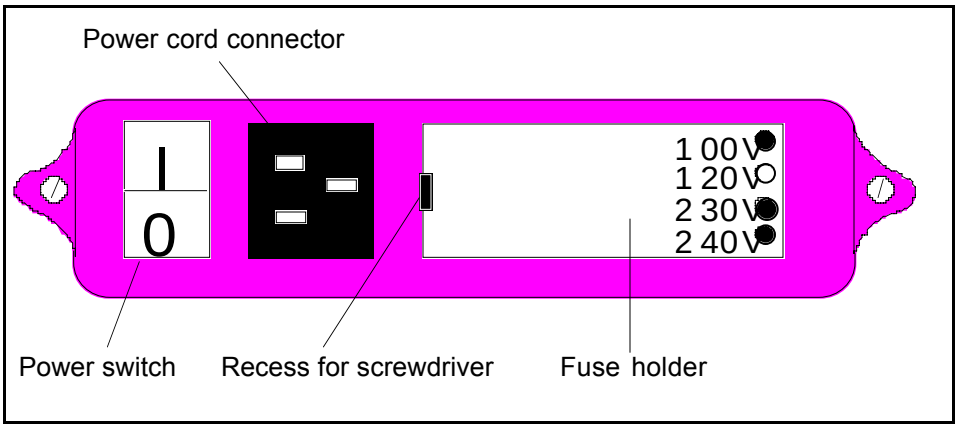
If liquid has entered the instrument below the microplate tray, push it into the measurement chamber (provided you have already cleaned it), and clean the bottom below the transport unit.

When using the injector unit (option)
If liquid has entered the area below the service door (either because the screw fittings at the injector head are not tight or due to leaky tubings), clean this area using a moist cloth. Before you open the service door, disconnect the MONOLIGHT 3096 from the wall outlet and open the instrument door!

8.2 Fuse Replacement (MONOLIGHT 3096)

The fuses on the instrument rear panel must be replaced when they are faulty or when changing the operating voltage (see 8.3). The fuses are in a black fuse holder next to the power cord connector (see Figure 8-1).

Figure 8-1: *Power supply unit on the instrument rear panel*



Proceed as follows

You need:

1 small flat head screwdriver
Spare fuses:
For operating voltage 230 V: Fuse: 160 mA slow-blow
For operating voltage 115/120 V: Fuse: 315 mA slow-blow
Use only UL-approved fuses!

CAUTION

❏ Turn power switch to off ("**0**").

❏ **Unplug the power cord from the wall outlet**.

❏ Insert a screwdriver or a similar tool into the recess on the right side of the fuse holder, push slightly and take the fuse holder out. You will see two fuses.

❏ Take the faulty fuse(s) out. ***Note****: You need both fuses for operation*. Faulty fuses can typically be detected by a broken fuse wire in the glass envelope.

- ❏ Insert new fuse(s).
- ❏ **Caution: Observe the required fuse types, current and fuse ratings. (see label on instrument rear panel). Otherwise, the operating safety cannot be guaranteed and the instrument will lose its UL (Underwriters Laboratories) approval.**
- ❏ Install fuse holder correctly, so that the pin fits into the hole corresponding to the operating voltage.

Function check:

- ❏ Attach power cable to the wall outlet.
- ❏ Turn instrument on.
- ❏ Check if green LED is on.

If the green LED is not on, please contact our Service Department.

8.3 Changing the Operating Voltage for MONOLIGHT 3096

You need:
1 small flathead screwdriver
1 flat-nose pliers
2 fuses for the respective operating voltage
For operating voltage 230 V: Fuse: 160 mA slow-blow
For operating voltage 115/120 V: Fuse: 315 mA slow-blow
Use only UL-approved fuses!

The instrument is designed for operation at 230 V (Euro voltage) or 115 V (US Voltage). The voltage setting must be changed only when operating the instrument in a country with a different operating voltage.

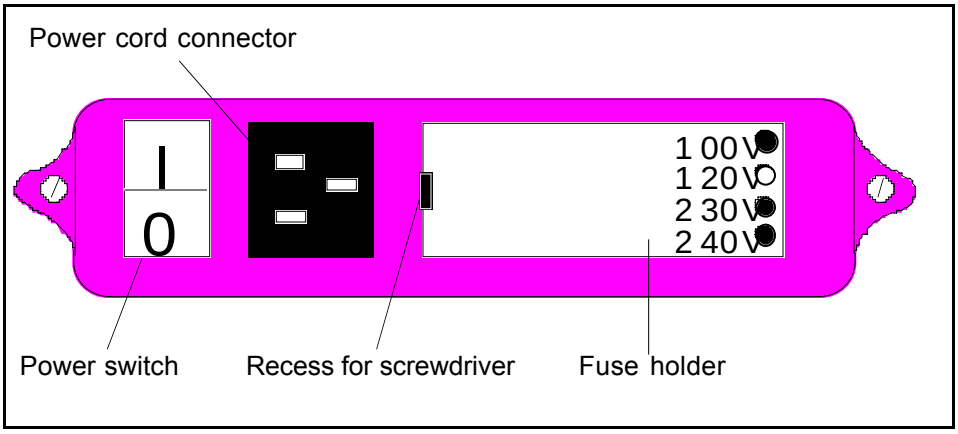
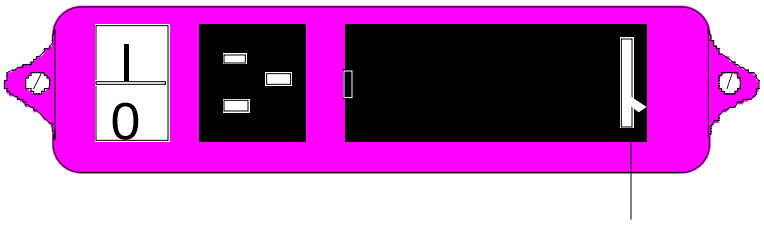


Figure 8-2: *Power supply unit on the instrument rear panel*

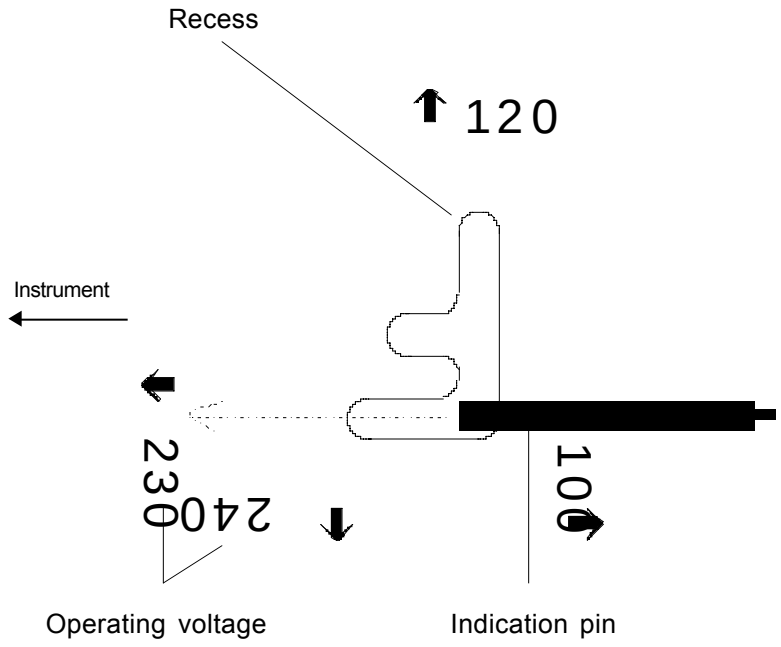
- ❏ Turn main switch to off ("**0**").
- ❏ **Unplug power cord from wall outlet**.
- ❏ Insert a screwdriver or a similar tool into the recess on the right side of the fuse holder, push slightly and take the fuse holder out.
- ❏ Replace both fuses and carefully put the fuse holder aside.

Figure 8-3:
Power supply unit with fuse holder taken off



- ❏ Remove the fuse holder to gain access to the voltage selection card. Pull out the voltage selection card horizontally, using a small pair of pliers to hold it at the white pin.
- ❏ Four operating voltages are printed on the voltage selection card and labeled accordingly. Take the pin out and position it in the recess, so that it is on one line with the correct operating voltage (see Figure 8-4).

Figure 8-4:
Voltage selection card



To change the operating voltage to 120V, you must position the indication pin as illustrated in this drawing and insert the card in this position again, pushing it in the direction of the arrow (printed side facing the power cord connector).

- ❏ Insert the voltage selection card as shown in Figure 8-4, pushing it into the instrument in the direction of the arrows, with the printed side facing the power cord connector.
- ❏ Adjust the indication pin in horizontal position.
- ❏ Insert fuse holder again properly so that the indication pin fits into the hole of the fuse holder with the proper operating voltage. It must work smoothly, otherwise the operating voltage is not set correctly.

Function check:

- ❏ Attach power cable.
- ❏ Turn instrument on.
- ❏ Check if green LED is on.

If the green LED is not on, please contact our Service Department.

Use only UL-approved fuses. Otherwise, the operating safety cannot be assured and the instrument will lose its UL approval.

8.4 Taking the Instrument out of Service (for Transport)

- ❏ Move microplate transport unit to home position (for example, by turning the Microplate Luminometer off and on.
- ❏ Open instrument door.
- ❏ Screw in red transport securing screw, pass it through the instrument frame into the drilled hole in the microplate transport unit to arrest the transport unit.
- ❏ Close instrument door.
- ❏ Turn luminometer off and disconnect it from main.
- ❏ Put luminometer in the original shipping cardboard box with the two foamed inserts. Additional cardboard boxes including foam inserts are available for purchase. Contact BD Biosciences Pharmingen, Technical Services, at 1-800-825-5832. Luminometer packing material Cat. No. is 551484, and the Injector Unit packing material is Cat. No. 551485.

8.5 Servicing and Cleaning the Injector System

This section describes service and maintenance work that can be carried out by the user on parts of the injector system, including the MONOLIGHT 3096 injector unit, the injector tubings to and from the MONOLIGHT 3096, and the injector tips in the MONOLIGHT 3096.

8.5.1 Regular Maintenance and Checks

- After use, rinse the pump thoroughly with distilled or deionized water (Section 8.7).
- If the pump is not used for a short time, it should be primed with distilled or deionized water.
- Do not operate the pump without liquid.
- Do not bend the injector tubings.
- Check screw fittings of injector tubings regularly for leaks (at the injector head and at the pump head). If any leaks occur, determine the cause (loose or defective screw fitting). Tighten or replace fitting (see section 8.10).
- Check injector pumps for leaks. If liquid escapes below the pump head, tighten syringe. If liquid escapes at the syringe, replace the syringe (section 8.12). If there is a leak in the pump head, you must replace the pump (contact Service).

8.5.2 Cleaning the Injector Unit (outside)

The surface of the injector unit is protected by a sturdy, washable varnish. If it is dirty, or dusty, clean it using a moist cloth. If necessary, use a mild household cleaner (e.g. detergent, but never scrubbing powder!).
If liquid gets inside the instrument, turn the pump off, open the locking door and clean the dirty parts. Determine the cause of the leak (screw fitting not tight or pump defective) and take the appropriate countermeasures (tighten or replace the fitting screw, see section 8.10).

8.6 Priming/Washing/Cleaning the Injector Tubings

Washing and priming of the injector tubings are controlled via the **Simplicity PC** software.

To clean the injector tubing system you should rinse it regularly with distilled or deionized water. Further recommendations for cleaning see section 8.8**.**

For measurement injections you must first prime the tubing, so that the full volume is injected with the first shot.

Proceed as follows to wash or prime the tubing systems:

1. Initialize the injector unit

- ❑ Select any measurement protocol that contains a measurement programmed with the respective injectors; click <**Run**> in the **Protocol Manager**.
- ❑ Click the Initialize button in the measurement menu. ()
- ❑ This opens a window prompting you to place the plastic tub in the microplate loading compartment (instead of a microplate) to collect any escaping liquid.
- ❑ As soon as you have positioned the plastic tub, click <**OK**>. The end of the initialization is indicated in another window.

2. Washing and Priming the Injector Tubings

- ❑ Make sure there is still enough space in the plastic tub for the escaping wash fluid.
- ❑ Click the Wash button to open the **Parameters for Priming Injectors** dialog box. ()
- ❑ Select the injector (**Inj. 1** or **Inj. 2**).
- ❑ Enter the volume/stroke (**ml**) and the number of strokes for washing and priming.
- ❑ The programmed procedure starts as soon as you click <**OK**>.

You may stop a started wash cycle any time by clicking the Stop Priming button. ()

8.7 Cleaning MONOLIGHT 3096 Injector Pumps and Tubings

The MONOLIGHT 3096 is equipped with a syringe injector system and Teflon tubings. All materials coming into contact with reagents are chemically resistant.

Daily routine cleaning procedure:

Use deionized or distilled to remove the reagents that are still inside the injection system.

Place the priming container on the microplate loading area. Start your measurement protocol, then initialize the injector(s) (at the end of a measurement this step may be skipped.)

1. Click on the <**Priming**> button and select the injector(s) you want to clean.
2. Select a volume of 500 µl and 10 injection cycles (strokes).
3. Click <**OK**> to start the wash process.

8.8 Basic Cleaning Before or After Long Breaks

Acid/Base Cleaning Procedure

This basic cleaning must be done before or after the injector has not been in operation for a longer period of time.

To keep the fluids for about 10 minutes in the syringes, move the syringe pistons to the extended position and hold them there. To do this you must use a cleaning protocol.
Therefore, create your own cleaning protocol from the protocol you are using for measurement: set the injector volumes as 150 µl and then save the protocol under another name (e.g., cleaning-protocol_acb).

Proceed as follows:

1. Wash with deionized or distilled water

1.1 Use deionized or distilled water to remove the reagents remaining in the injection system.
1.2 Place the priming container on the microplate loading area.
1.3 Start the cleaning protocol and initialize the injectors.
1.4 Click the <**Priming**> button and select the injector(s) you want to clean.
1.5 Select a volume of 150 µl and 20 injection cycles (strokes).
1.6 Click <**OK**> to start the wash process.
1.7 Repeat steps 1.4 - 1.6.

2. 1st Cleaning Step:
Cleaning the Injector(s) with 0.1N NaOH

2.1 Remove front cover of injector unit to watch the syringe movement.
2.2 Fill the reagent bottles and injector tubing with 0.1N NaOH.
2.3 Click the <**Priming**> button and select the injector(s) you want to clean.
2.4 Select a volume of 150 µl and 20 injection cycles (strokes).
2.5 Click <**OK**> to start the cleaning process.
2.6 Repeat steps 2.3 – 2.5
2.7 Start a measurement by clicking the green <Start> button; click the red <Stop> button when the syringe has reached the extended position, and let the syringe stay in this position for about 10 minutes.
2.8 Wash the syringe with deionized or distilled water using the priming routine (20 cycles (strokes) with 150 µl each).

3. 2nd Cleaning Step: Cleaning with 0.1N HCL

3.1 Fill the syringe with 0.1N HCL and leave this solution for about 10 minutes in the syringe (same as for cleaning with NaOH, steps 2.2 – 2.6).
3.2 Start a measurement by clicking the green <**Start**> button; click the red <**Stop**> button when the syringe has reached the extended position, and let the syringe stay in this position for about 10 minutes.
3.3 **Then wash the syringe with deionized or distilled water**, using the priming routine (20 cycles (strokes) with 150 µl each).

8.9 Promega Reagents Cleaning Procedure

One of the Promega Dual Luciferase Assay reagents, the Stop & Glo Reagent, has a slight affinity to plastic materials. To avoid cross contamination when changing the injector tubings, we recommend the follow the cleaning procedure outlined below.
To keep the fluids in the syringes for about 30 minutes, move the syringe pistons to the extended position and hold them there. To do this you must use a cleaning protocol.
Therefore, create your own cleaning protocol from the protocol you are using for measurement: set the injector volumes as 150 µl and then save the protocol under another name (e.g. cleaning_DLR_Assay).

Proceed as follows:

1. Wash with deionized or distilled water

1.1 Use deionized or distilled water to remove the reagents remaining in the injection system.
1.2 Place the priming container on the microplate loading area.
1.3 Start the cleaning protocol and initialize the injectors.
1.4 Click the <**Priming**> button and select the injector(s) you want to clean.
1.5 Select a volume of 150 µl and 20 injection cycles (strokes).
1.6 Click <**OK**> to start the wash process.
1.7 Repeat steps 1.1 - 1.6.

2. Cleaning with Ethanol

2.1 Fill the reagent bottles and injector(s) with 70% Ethanol.
2.2 Remove front cover of injector unit to watch the syringe movement.
2.3 Click the <**Priming**> button and select the injector(s) you want to clean.
2.4 Select a volume of 150 µl and 20 injection cycles (strokes).
2.5 Click <**OK**> to start the wash process.
2.6 Repeat steps 2.3 – 2.5.

3. Re-washing with deionized or distilled water.

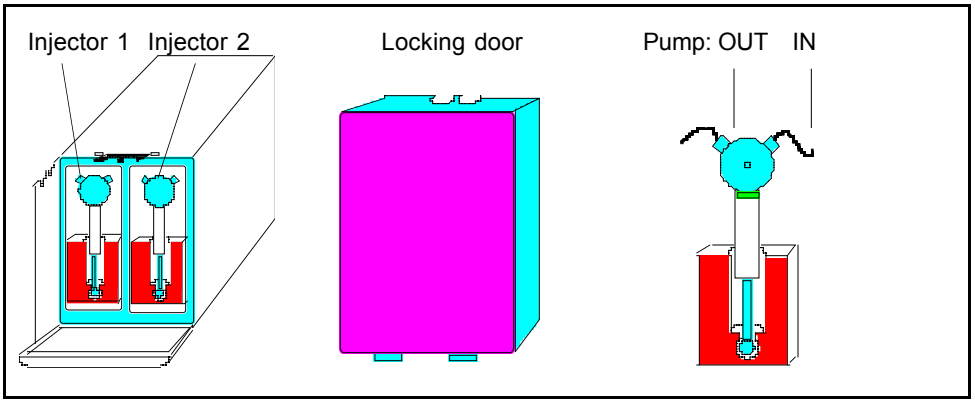
3.1 Wash the syringe with deionized or distilled water, using the priming routine (steps 1.1 – 1.7) (20 cycles (strokes) with 150 µl each).
3.2 Repeat the wash process with deionized or distilled water to remove all traces of ethanol.

8.10 Establishing Tubing Connections

If a tubing connection (at the pump head or at the injector head) is faulty, or to replace an injector tubing, you must provide a new tubing connection. To this end, additional injector tubings, fitting spare parts and a special tool for expanding the tubings are supplied as extras.

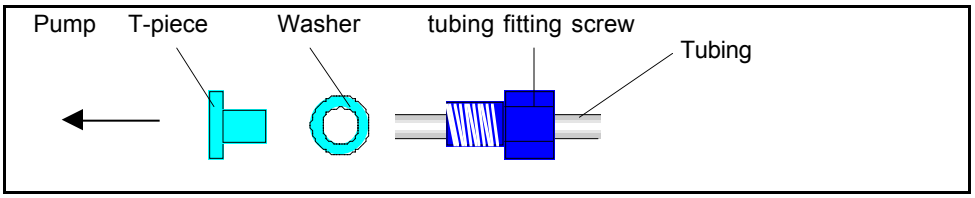
a) Connection at pump head

Figure 8-7: *Injector unit*



- ❑ Turn injector unit off.
- ❑ Pull off the locking door of the injector unit vertically from above.
- ❑ Push tubing fitting screw over the tubing such that the threading is facing the tubing end. Then push a washer over the tubing. (Figure 8-8).
- ❑ Using the special tool supplied, expand the tubing end by pushing the tubing over the tool's arbor.

Figure 8-8: *Manufacturing the tubing connection*



- ❑ Push T-piece into expanded tubing end.
- ❑ Insert tubing end with T-piece into threaded borehole of pump head, turn fitting screw with washer into the threading of the pump head and finger-tighten it.

b) Connection at injector head

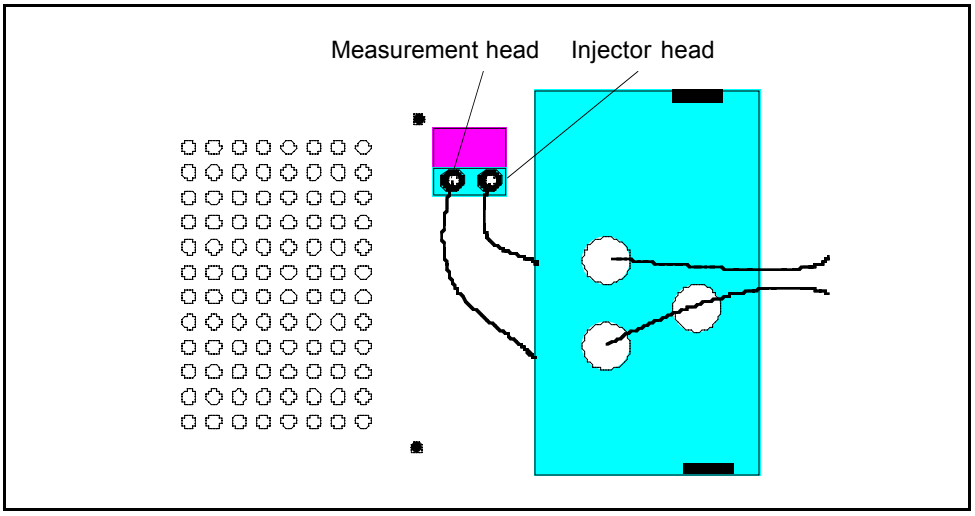


Figure 8-9: *MONOLIGHT 3096 open*

- ❑ Turn MONOLIGHT 3096 off and disconnect it from the wall socket.
- ❑ Open instrument door of MONOLIGHT 3096, open screws of service door and take door off (Figure 8-9).
- ❑ Push tubing fitting screw over the tubing such that the threading is facing the tubing end (Figure 8-10). Then push a washer over the tubing.
- ❑ Using the special tool supplied, expand the tubing end by pushing the tubing over the tool's arbor.

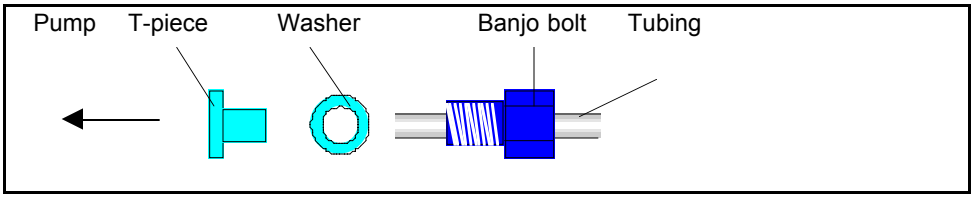


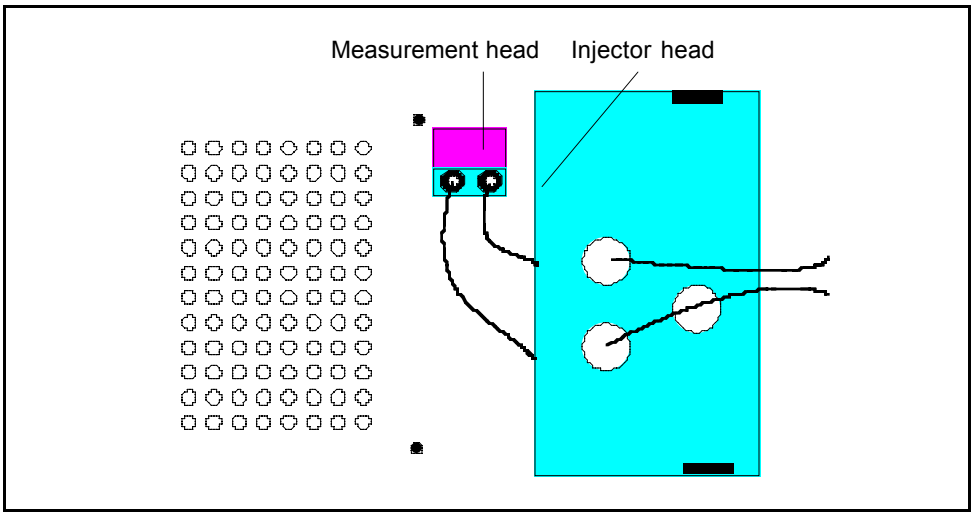
Figure 8-10: *Manufacturing the tubing connection*

- ❑ Push T-piece into expanded tubing end.
- ❑ Insert tubing end with T-piece into threaded borehole of pump head, turn fitting screw with washer into the threading of the pump head and finger-tighten it.

8.11 Replacing Injector Tips

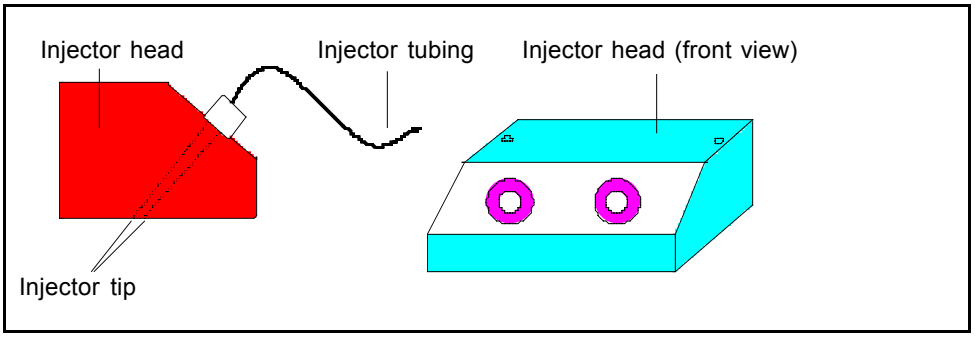
The injector tips are located in the injector head behind the service door of the **MONOLIGHT 3096**.

Figure 8-11: *MONOLIGHT 3096 open*



- ❑ Turn MONOLIGHT 3096 off and disconnect it from wall socket.
- ❑ Open instrument door of MONOLIGHT 3096, open screws of service door and take door off.
- ❑ Open the 2 fixing screws of the injector head and take off the injector head directly behind the measurement head.

Figure 8-12: *Position of injector tip in injector head*



- ❑ Unscrew the respective tubing fitting screw (made of metal) with tubing and pull it out.
- ❑ Push out injector tips towards the top.
- ❑ Insert new injector tip and screw fitting screw into the injector head (see also the previous section 8.10).

- ❑ Insert injector head (the sloping part is facing you). Fit the injector tips into the boreholes in the MONOLIGHT 3096 and the adjusting bolts in the recesses in the injector head (Figure 8-13).
- ❑ Insert fixing screws and tighten them.
- ❑ Close service door properly (light-tight) (see also page 20).

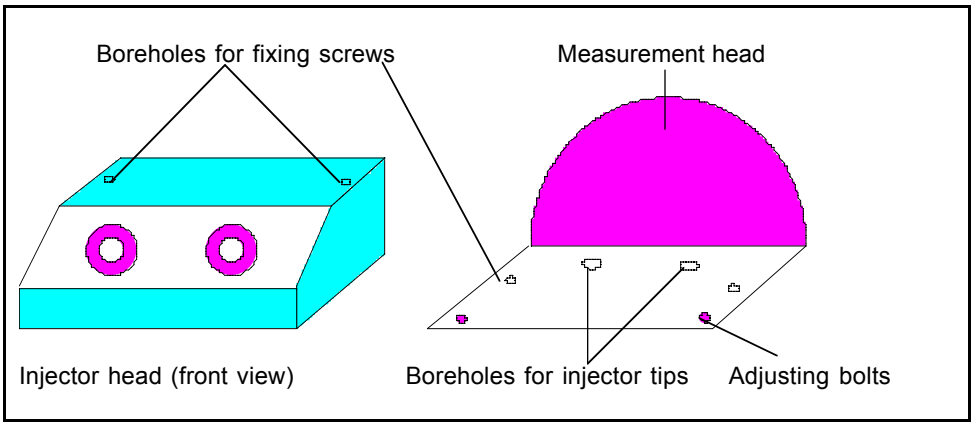
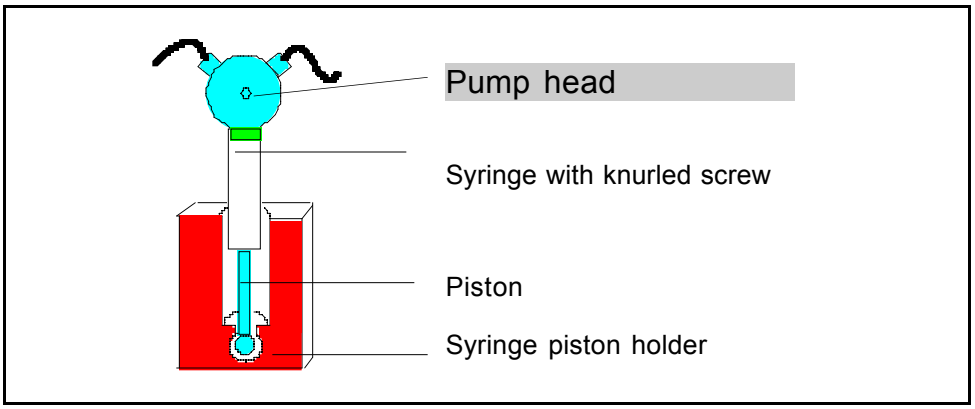


Figure 8-13: *Installing the injector head*

8.12 Replacing Pump Syringes

When the pump syringe is defective (e.g. leaky), you must replace it.

Figure 8-14: *Design of Cavro pump*

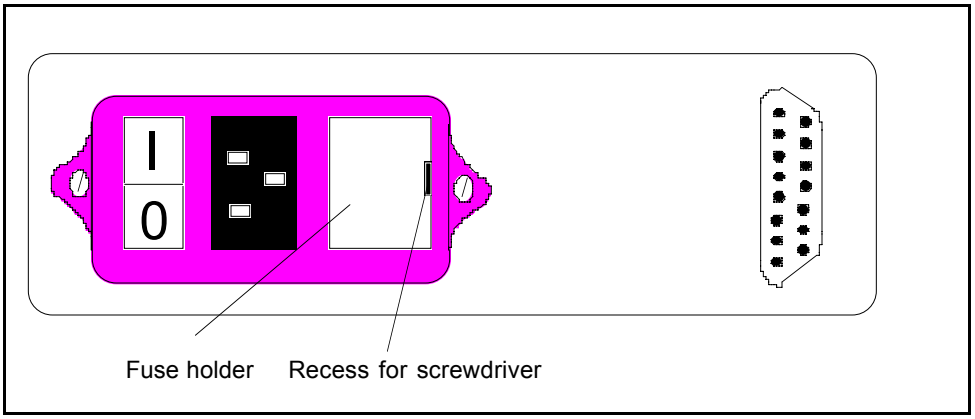


- ❑ Empty pump by running a wash cycle with several strokes without having a bottle with liquid connected (see section 0)
- ❑ Turn injector unit off and disconnect it from wall socket.
- ❑ Pull of locking door of injector unit vertically from above.
- ❑ Open knurled screw at syringe until you can detach it from the pump head, and push it down with the syringe piston holder.
- ❑ Now you may take off the syringe from the front.
- ❑ Insert new syringe in the syringe piston holder, push it up and fix it with the knurled screw to the pump head.
- ❑ Close injector unit.

8.13 Replacing Fuses in the Injector Unit

The fuses on the instrument rear panel must be replaced when they are faulty or when changing the operating voltage (see 0). The fuses are stored in a black fuse holder next to the main plug (see Figure 8-5).

Figure 8-5: *Power supply unit on the instrument rear panel*



Proceed as follows

You need:

1 small flathead screwdriver
Spare fuses:
For operating voltage 230 V: Fuse: 160 mA slow-blow
For operating voltage 115 V: Fuse: 315 mA slow-blow
Use only UL-approved fuses!

- ❑ Turn main switch to off position ("**0**").
- ❑ Disconnect power cord from wall outlet.
- ❑ Insert a screwdriver or a similar tool into the recess on the right side of the fuse holder, push slightly and take the fuse holder out. The safety holder includes one fuse each for operating voltage 115 V and 230 V.
- ❑ Remove the faulty fuse. Faulty fuses can typically be detected by a broken fuse wire in the glass envelope.

- ❑ Insert new fuse(s) and observe the required current and fuse ratings. Otherwise, the operating safety cannot be guaranteed and the instrument will lose its UL (Underwriters Laboratories) approval.
- ❑ Install fuse holder correctly, so that proper operating voltage is indicated.

Function check:

- ❑ Connect power cord to wall outlet.
- ❑ Turn instrument on.
- ❑ Check if green LED is on.

If the green LED is not on, please contact our Service Department.

8.14 Changing the Operating System Voltage in the Injector Unit

You need:
1 small flathead screwdriver
Spare fuses

For operating voltage 230 V: Fuse: 160 mA slow-blow
For operating voltage 115 V: Fuse: 315 mA slow-blow
Use only UL-approved fuses!

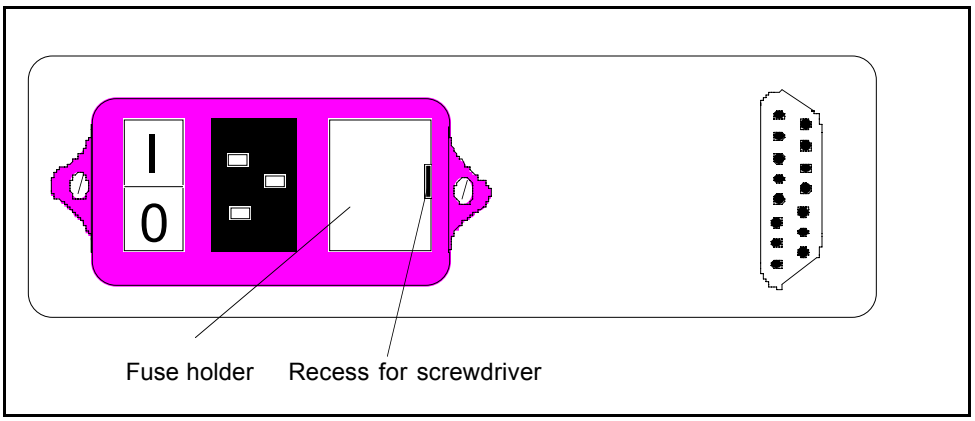


Figure 8-6: *Power supply unit on the rear panel of the injector unit*

- ❑ Turn main switch to off position (**0**).
- ❑ Disconnect power cord from wall outlet.
- ❑ Insert a screwdriver or a similar tool into the recess on the right side of the fuse holder, push slightly and take the fuse holder out. The safety holder includes one fuse each for operating voltage 115 V and 230 V.
- ❑ Turn the fuse holder by 180° and insert the fuse holder again such that the label indicating the respective operating voltage is visible in the window.

9. Technical Data

MONOLIGHT 3096 Microplate Luminometer	
Sample format	96 well microplate, non-transparent, fixed or strip format
Detector	Photomultiplier with bialkali cathode (300 - 650 nm)
Sensitivity	Less than 50 attomol ATP, depending on reagents
Crosstalk	Less than 3×10^{-5} with 96 well microplates
Dynamic range	More than 6 decades
Hardware	Microcontroller for control of all instrument functions
Injector Ports	2 at measurement position and 2 in the neighboring well positon
Measuring Time	About 100 s per microplate, depending on the measurement protocol
Scan Pattern	Random selectable single well, access by mouseclick
Comm Ports	Serial interface RS232 port for PC, CAN-bus connection to injector unit
Dimensions	W 400 mm, D 545 mm, H 200 mm
Weight	Approx. 17 kg
Operating Voltage	230 V 50 Hz; 115 V 60 Hz
Power Consumption	35 VA
Temp Range	Storage 0° - 40°C; Operation 10° - 35°C
Humidity	10% - 80% (no condensation)
Regulatory	CE

Injection Unit	
Number of Injectors	2 injectors
Injection Volumes	10–150 µl per group
Tubing	Chemically inert PTFE tubing and connections (PTFE or KEL-F), inter-changeable liquid handling system and tips
Dimensions	W 158 mm, D 330 mm, H 190 mm
Weight	3 kg
Power	230 V 50 Hz; 115 V 60 Hz
Power Consumption	25 VA
PC Software	
Required Hardware	IBM compatable PC, one available serial port
Screen Resolution	640 x 480
Colors	Min. 16
RAM	Min. 8 MB
Operating System	Windows® 95, Windows® 98, Windows® NT 4.0
Optional Software	Microsoft® Excel 97
Standard Configuration	Protocol Manager, Raw Data
Available Additions	Fast Kinetics, Slow Kinetics
Windows® Features	Fully resizable grids, non–contiguous areas selectable, multiple document interface, resizable, zoomable graphs, status bar, draggable tool bars, etc.